Aspects of palliative chemotherapy for lung cancer

Smit, Egbert Frederik

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E.F. Smit

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Stellingen

1. Het bereiken van hoge leeftijd is een risicofactor voor inadequate behandeling van het (bronchus-)carcinoom.

2. Carboplatin is een nefrotoxisch cytostaticum.

3. Om de effectiviteit van tweede lijns chemotherapie op juiste waarde te schatten dient het begrip tumorresistentie ook klinisch, dat wil zeggen operationeel, gedefinieerd te worden.

4. Continue infusie van carboplatin vergroot de therapeutische index van dit cytostaticum.

5. Cellijnen zijn door hun genetische instabiliteit slecht bruikbaar voor het voorspellen van door cytostatica geïnduceerde tumorrespons in de individuele patiënt.

6. Hersenmetastasen van een kleincellig bronchuscarcinoom dienen te worden opgevat als uiting van een systematische ziekte. In deze situatie is systemische behandeling meer op haar plaats dan lokale behandeling.

7. Het bereiken van een "complete response" is een overschatting van de (chemo-) therapeutische mogelijkheden bij de behandeling van longcarcinomen.

8. Promovendi promoveren promotores et vice versa.

9. I never fully understood how these cells managed to gain access into the CNS, and then after getting themselves in, relocked the door to prevent access to chemotherapy. (Andrew T. Turrissi. J.Clin.Oncol. 8:196-199, 1990)

10. Het verdient aanbeveling de term milieutechnologie te vervangen door vuilnisbelt-technologie.

11. Men kan niet tegelijkertijd mensen genezen en alles begrijpen. Laten we daarom zo vlug mogelijk genezen. Dat heeft het meest haast. (In: De pest, Albert Camus)

12. Er bestaat evenredige relatie tussen de mate van eiwitbinding van platinumderivaten en de door deze derivaten veroorzaakte nefrotoxiteit na intraveneuze toediening.

13. De effectiviteit van langdurige toediening topoisomerase II remmers, zoals etoposide, dient te worden getest in tumoren met een lage mitotische index.

RIJKSUNIVERSITEIT GRONINGEN

Aspects of palliative chemotherapy
for lung cancer

Proefschrift

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus Dr. L.J. Engels
in het openbaar te verdedigen op woensdag 3 oktober 1990
des namiddags te 4.00 uur

door

Egbert Frederik Smit

geboren op 24 mei 1961 te Winschoten

| Promotores | : | Prof. Dr. G.H. Koëter |
| | | Prof. Dr. G.K. van der Hem |

Referenten	:	Dr. E.G.E. de Vries
		Dr. P.E. Postmus
		Dr. N.H. Mulder

CONTENTS

VOORWOORD

Dit proefschrift is tot stand gekomen binnen het samenwerkingsverband van de onderaf-
deling Longziekten (Hoofd: Prof. Dr. G.H. Koëter) en de Werkgroep Interne Oncologie
(Dr. D.Th. Sleijfer, Dr. N.H. Mulder) van de afdeling Interne Geneeskunde (Hoofd: Prof.
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Tisna gaf mij de rust en steun aan dit proefschrift te werken.

INTRODUCTION

Lung carcinoma is the main cause of cancer related deaths among the male population in The Netherlands. In females this carcinoma is only surpassed by breast and colon cancer. In The Netherlands in 1987, 8.500 persons died due to lung carcinoma (1). It is anticipated that despite government-installed preventive measures -including stop-smoking programs- the number of persons suffering from lung cancer will continue to rise in the near future (2,3). The results of all therapeutic modalities for lung cancer have reached a plateau for many years with about 10% overall cure (4). For therapeutic reasons, lung cancer is generally divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). For NSCLC surgery is the only curative modality for patients without demonstrable metastatic disease (5). For SCLC, which comprises 20-25% of all cases, combination chemotherapy forms the cornerstone of therapy with the vast majority of patients responding to therapy with prolonged survival and a small percentage of cures. While SCLC is responsive to chemotherapy, NSCLC is only moderately responsive to such therapy (6). Although much knowledge has been gathered on the biology of lung cancer, this basic research did not, however, result in improved survival of patients treated by chemotherapy for lung cancer (7). The failure of chemotherapy to cure lung cancer patients is basically a problem of drug resistance. Several resistance mechanisms have been discovered in the recent years. The most widely studied form is the so called pleiotropic drug resistance (PDR) (8). PDR has emerged as a consistent mechanism of resistance for several structurally unrelated cytotoxic agents in cancer cells. Resistance seems to be caused by a decreased intracellular drug concentration, caused by an increased efflux pump. This efflux pump can be antagonated *in vitro* by calcium antagonists. It has become clear that reversal of resistance mediated by the PDR-phenotype is in some cases possible in the clinic (9,10). PDR however, seems to play little or no role in lung cancer (11). Resistance mechanisms which have been identified *in vitro* in lung cancer cell lines include enhanced DNA repair capacity and altered drug-topoisomerase interactions (12). For these and other drug resistance mechanisms it has by no means been elucidated whether they can be circumvented by sophisticated measures in the near future.

How then can the fate of patients with this common malignancy be influenced positively? Until now, the only way to improve treatment results, as far as response rates are concerned, is by dose intensification of the chemotherapy. The use of haematopoietic growth factors might in the near future facilitate such regimen (13). The subgroup of patients suffering from lung cancer with "favorable" prognostic features are probably those who are able to withstand more aggressive forms of chemotherapy. This small group includes patients with stage III[A] NSCLC and limited disease SCLC. On the other hand a considerable group of patients remains that is unlikely to benefit enough from treatment intensification to justify its inherent toxicity. These are patients with metastatic NSCLC, elderly SCLC patients, patients with extensive disease SCLC and patients with a relapse of SCLC. Therefore, for the majority of patients with lung cancer the main goal of treat-

ment will be adequate palliation. Improvement in the fate of these patients should be sought in amelioration of side effects of treatment ideally with preservation of present days response rates. In this thesis several attempts are described to achieve this goal. The aims of the study were to establish chemotherapy regimen for such patients with equal anticancer activity but less toxicity (chapters 1-7), to reduce toxicity by manipulating the pharmacology of cytotoxic drugs (chapters 8,9) and *in vitro* (pretreatment) selection of patients who are most likely to benefit from cytotoxic treatment (chapters 10,11).

Chapter 1 reviews aspects influencing treatment outcome of elderly patients suffering from cancer, with special emphasis on SCLC.

In chapter 2 the results of a phase II study with a potentially less toxic regimen for elderly patients with SCLC are reported.

The congener of etoposide, teniposide, may have more efficacy against SCLC (14). Because of the known schedule dependency of its activity it is attractive to use a regimen with multiple daily infusions. If given as an out-patient treatment, this is rather troublesome for most patients. A way to overcome this inconvenience is the substitution of the intravenous drug by an oral preparation. In chapter 3 the results of a phase I study of oral teniposide are described.

Brain metastases are a frequent complication in the clinical course of SCLC (15). For decades whole brain radiotherapy has been considered to be the standard treatment in this situation. However, side effects of brain irradiation are considerable and survival in these patients remains very short. Furthermore, most patients will also need systemic treatment for synchronous extra-cranial tumor progression. Based on the experience of our own group (16,17), we conducted a phase II study of single agent teniposide (intravenous) in patients with brain metastases of SCLC (chapter 4).

One of the most widely used drugs for lung cancer is cisplatin, also probably the most toxic cytostatic drug in standard regimen. One of the new analogues of this drug -carbo-platin- is promising for the treatment of lung cancer. Carboplatin was selected for clinical use because of its presumed lack of nephrotoxicity (18). Chapter 5 and 5a evaluate the nephrotoxicity of carboplatin in 10 patients with lung cancer.

Cisplatin is often used in second line regimen for SCLC patients relapsing after induction chemotherapy. In chapter 6 this potential application of a carboplatin containing regimen was tested.

Chapter 7 contains the results of a phase II study using the same drug combination in patients with NSCLC.

Continuous infusion may be a way to enhance the therapeutic index -more efficacy and less toxicity- of cytotoxic drugs. In addition there are several theoretical advantages of this mode of drug administration compared to bolus infusion with regard to antitumor action, especially in solid tumors (19). Chapter 8 provides details of a phase I and pharmacokinetic study of continuous infusion of carboplatin for 21 days.

Vindesine is arguably one of the most active drugs in NSCLC. In chapter 9 a similar study with vindesine is described.

Currently (second line) chemotherapy for lung cancer is given on a trial and error base. A major step forward would be the selection of patients for treatment after *in vitro* chemosensitivity testing. This would constitute a way to tailor a patient's therapy individually and thereby avoiding unnecessary toxicity of chemotherapy. We investigated if the *in vitro* response of small cell lung cancer cell lines to cytotoxic drugs correlates with the clinical data of the patients they were derived from (chapter 10).

A prospective study of predictive testing *in vitro* on fresh human lung cancer biopsies is the subject of chapter 11.

The results of our studies are summarized in chapter 12 and potentially new directions are discussed.

REFERENCES

1.   Coebergh JWW. Epidemiologie van kanker. In: Grobbee DE, Hofman A. (red). Epidemiologie van ziekten in Nederland. Utrecht, Bunge, 1989; 124-140.

2.   Verbeek AML, Peeters PHM, Sturmans F. Is de top van de longkanker-epidemie in Nederland in zicht? Ned T Geneesk. 129:2365-2369, 1985.

3.   Brown CC, Kessler LG. Projections of lung cancer mortality in the United States 1985-2025. J. Natl. Cancer Inst. 80:43-51, 1988.

4.   Stjernsward J, Stanley K. Lung cancer - a world wide health problem. Proc. 5th world conference on lung cancer 1988:p11-14.

5.   Mountain C. Therapy of stage I and II non-small cell lung cancer. Semin. Oncol. 10:71-75, 1983.

6.   Minna JD, Pass H, Glatstein E, Ihde DC. Cancer of the lung. In: DeVita VT, Hellman S, Rosenberg SA (eds). Cancer. Principles and practice of oncology. 3rd ed. Philadelphia, J.P. Lippincott Inc., 1989: 591-705.

7.   Beck LK, Kane MA, Bunn PA. Innovative and future approaches to small cell lung cancer treatment. Sem. Oncol. 15:300-314, 1988.

8.   Rothenberg M, Ling V. Multidrug resistance: molecular biology and clinical relevance. J. Natl. Cancer Inst. 81:907-910, 1989.

9.   Dalton WS, Grogan TM, Meltzer PS, Scheper RJ, Durie BGM, Taylor CW, Miller TP, Salmon SE. Drug resistance in multiple myeloma and non-hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. J. Clin. Oncol. 7:415-424, 1989.

10. Fojo T, McAtee N, Allegra C, Bates S, Mickley L, Keiser H, Linehan M, Steinberg S, Tucker E, Goldstein L, Gottesman MM, Pastan I. Use of quinidine and amiodarone to modulate multidrug resistance mediated by the mdr1 gene. Proc. ASCO:262, 1989.

11. Lai SL, Goldstein LJ, Gottesman MM, Pastan I, Tsai CM, Johnson BE, Mulshine LJ, Ihde DC, Kayser K, Gazdar AF. MDR1 gene expression in lung cancer. J. Natl. Cancer Inst. 81:1144-1150, 1989.

12. Zijlstra JG, de Vries EGE, Mulder NH. Multifactorial drug resistance in an adriamycin-resistant human small cell lung carcinoma cell line. Cancer Res. 47:1780-1784, 1987.

13. Bronchud MH, Scarffe JH, Thatcher N, Crowther D. Phase I/II study of recombinant human granulocyte colony-stimulating factor in patients receiving intensive chemotherapy for small cell lung cancer. Br. J. Cancer 56:809-813, 1987.

14. Bork E, Hansen M, Ersbol J, Dombernowsky P, Bergmann B, Hansen HH. A randomized stu-
     dy of teniposide (Vm-26) versus Vepeside (Vp-16) as single agents in previously untreated
     patients with small cell lung cancer (SCLC) >70 years. Proc. ASCO, 890, 1989.
15. Newman SJ, Hansen HH. Frequency, diagnosis, and treatment of brain metastases in 249 con-
     secutive patients with bronchogenic carcinoma. Cancer 33:492-496, 1974.
16. Postmus PE, Haaxma-Reiche H, Vencken LM, Meinesz AF, Sleijfer DTh, Mulder NH.
     Remission of brain metastases from small cell lung cancer after high-dose chemotherapy.
     Ann. Intern. Med. 101:717, 1984.
17. Postmus PE, Haaxma-Reiche H, Sleijfer DTh, Kirkpatrick A, McVie JG, Kleisbauer JP & the
     EORTC Lung cancer cooperative group. High dose etoposide for brain metastases of small
     cell lung cancer. A phase II study. Br. J. Cancer 59:254-256, 1989.
18. Harrap KR, Jones M, Wilkinson CR, Clinck HM, Sparrow S, Mitchley BCV, Clarke S,
     Veasey A. Antitumor, toxic and biochemical properties of cisplatin and eight other platinum
     complexes. In: Cisplatin current status and new developments. Prestayko AW, Crooke ST,
     Carter SK (eds). Academic Press, London 1980: 193-212.
19. Vogelzang NJ. Continuous infusion chemotherapy: a critical review. J. Clin. Oncol 2:1289-
     1304, 1984.

SMALL CELL LUNG CANCER IN THE ELDERLY. FACTORS INFLUENCING THE RESULTS OF CHEMOTHERAPY: A REVIEW

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INTRODUCTION

As one ages, the risk of the development of malignant disease rapidly increases, resulting in a disproportionately higher frequency of the occurrence of cancer in the elderly population. Indeed, more than 50% of all cancers are diagnosed in patients over the age of 65 years, despite the fact that this group compromises only 10% (USA) - 15% (The Netherlands) of the population at large. In most western countries the number of persons in this age category is still increasing and it is predicted that by the turn of the century about 20% of the population will be older than 65 years (1, 2).

The incidence of most cancers increases sharply with age, and lung cancer appears to be no exception to this rule (3-6). For example, among male lung cancer patients in The Netherlands 61% were 65 years or older and 21% were more than 75 years of age at the time of diagnosis. For females these figures were 45% and 15%, respectively. Also, the incidence of lung cancer will continue to increase in the near future, although in some industrialized countries in males the incidence in the younger age groups is falling (3, 8, 9).

The incidence of all histologic types of lung cancer peaks at 70-74 years of age for males and at 65-69 years for females (9). About 25% of these patients have a clinically distinct form of lung cancer, namely small cell lung cancer (SCLC) (10). The incidence of this most rapidly progressive form of lung cancer seems to have increased during the last decades (11-14).

SCLC is sensitive to a wide range of chemotherapeutic agents and chemotherapy has extended survival four- to fivefold (9). However, definite cure is rare (15) because in most initially responding patients the tumor relapses and causes death within several months after the start of chemotherapy. Mostly, with chemotherapy in SCLC, the best that can be obtained is a temporary relief of symptoms and therefore for most patients it is a palliative form of therapy. This has to be weighed against the detrimental effects of cytostatic treatment (16). Apparently, the balance between potential benefits and predictable side effects of treatment in elderly patients is such that physicians tend to offer the elderly patient with cancer fewer options for cytostatic treatment (17-20). According to Longo and Carbone

(21) this is caused by "a prejudice that the older patient is less able to tolerate the stresses of chemotherapy".

In this review we will discuss some aspects of chemotherapy in elderly patients with cancer, with special emphasis on SCLC.

2. CANCER, AGE AND PROGNOSIS

One of the key questions for a therapeutic decision concerning treatment of cancer in the elderly is whether age by itself is an adverse prognostic factor of survival. For some solid tumors, such as breast cancer, melanoma and thyroid cancer, survival declines with age (22), but most studies of prostatic cancer indicate that age is not a determinant of prognosis (23).

Whether age has a negative influence on survival of lung cancer patients in general is a matter of debate. Rossing et al (24) analyzed records of over 6000 patients with lung cancer. For the poorly defined subgroup of anaplastic tumors - including large cell carcinomas - age below 45 years was associated with significantly greater survival probabilities than for patients older than 55 years. Also, for adenocarcinomas and squamous cell carcinomas a survival advantage for patients of 45 to 54 years was seen compared to older patients (> 55 years), but not to patients younger than 45 years. Lanzotti et al (25), comparing 428 inoperable lung cancer patients, including 55 SCLC patients, saw that extensive disease (ED) patients older than 70 years had a significant worse median survival time (MST) regardless of histologic subtype. This was contradicted by the study of Coy et al (26). Using Feinstein's index age was not related to survival in 1271 unresected lung cancer patients.

2.1. Small cell lung cancer, age and prognosis
For SCLC also conflicting data in the literature are found. In a recent study, Poplin et al (27) compared 49 patients older than 65 years to 164 patients younger than 65 years. There were no significant differences between patient characteristics, although in the older age group the median performance status was slightly worse. The results showed that patients older than 65 years with either limited disease (LD) or ED had MST equal to their younger counterparts. Surprisingly, the youngest (< 55 years) patients with LD had shorter MST compared to older patients with LD. No such difference could be found for ED.

In contrast, in a series of 224 SCLC patients, Maurer et al (28) found the MST for the 28 patients older than 70 years to be less than half the MST of the patients younger than 70. This was caused by a much lower complete response (CR) rate for LD patients in the elderly, as MST of complete responders was not affected by age.

Several authors addressed the issue of age and survival in SCLC in various analysis models. In the largest series published today, Osterlind et al (29) analyzed prognostic factors in 778 patients with SCLC with an upper age limit of 70 years. Age was a negative

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prognostic factor in ED, but not in LD. However, in the first group of 214 patients reported by the same group (30) no such influence of age was seen. In the study of Feld et al (31) an adverse influence of age was seen. In 300 patients ranging from 35-80 years, younger age improved survival in a multifactorial model.

Ihde et al (32) found that age had no discernable association with survival in a group of 106 SCLC patients, with 16 older than 65 years. Also, Edmonson et al (33), Vincent et al (34) and Souhami et al (35) found age unrelated to prognosis in SCLC. Although published in abstract form only, Feld et al (36) noted that age had no significant influence on prognosis in a series of 480 patients. Finally, Kelly et al (37) compared a selected group of 34 patients older than 65 years (with 28 > 70 years) to 62 patients below 65 years, both groups receiving the same treatment. No difference in MST among the groups was seen.

As MST need not necessarily reflect long term survival (LTS) (38), it may be that elderly patients do not reach two or even five year survival as often as younger patients. Again, no conclusive statement can be made from the available literature. Johnson (39) reported on 14 patients alive after 5-11 years after treatment. Age did not differ significantly in LTS when compared to the 238 non-survivors. Niiranen et al (40) noted that the age of the 19 five year survivors was comparable with the whole study population (1019 patients). These results were contradicted by the extensive analysis of Osterlind et al (41) of 815 patients < 70 years. At eighteen months disease free survival age > 60 years was a negative pretreatment characteristic in ED. In a subsequent report (42), in 211 patients with CR (112 > 60 years) high age had a negative influence on response duration in ED. Both these observations held not true for LD in their material.

Maurer et al (28) saw no LTS in the older age group. Although long term survivors were predominantly younger than 60 years, this difference was not significant. Sierocki (43) noted a slightly younger median age, 54 versus 60 years, in the group of patients surviving for more than 12 months after initiation of treatment.

A realistic estimate of the effect of age per se on the prognosis of elderly patients with SCLC is hampered by several factors. First, extent of disease, initial performance status and weight loss are the main determinants of survival (44). Second, because patients > 70 years have often been and still are excluded from clinical trials, for this group of patients inferences hardly can be made. Finally, because in all studies data from patients already accepted for treatment are analyzed retrospectively, by this approach a bias concerning elderly patients may have been introduced. This is supported by the observation that the elderly patient with cancer tends to be underrepresented in chemotherapeutic trials compared to epidemiologic data (17, 20, 45). Therefore, systematic selection in favour of elderly patients without other well-known adverse prognostic features may be present in these studies.

On the other hand, several factors may influence survival in elderly patients with SCLC. As the prevalence of chronic diseases rises with age, many of these patients also suffer from intercurrent and concurrent medical problems. These will certainly compromise the patient's ability to withstand the effects of cancer and treatment interventions. To date it is not known if an elderly patient with SCLC who is not suffering from such medical complications has the same prognosis after treatment as younger patients.

3. AGE AND TUMOR BIOLOGY

Also, there might be a difference in biological behaviour of certain tumors with ageing. In general, tumors tend to be as localized in younger as in old patients (22). Cervix and colon carcinomas are more likely to be advanced with increasing age (23). Interestingly, for lung carcinoma there seems to be an inverse relationship between stage of disease and age (10, 46, 47). In the recent study of Teeter et al (10) the trend towards a more localized disease for females was small and significant only for squamous cell carcinoma. In males significant trends were noted for all histological types, except for large cell carcinoma. The proportion of localized disease increased with increasing age from 21% in the youngest (40-49 years) group to 44% in the 80+ age group. Again, squamous cell carcinoma displayed the most obvious trend (25 versus 55%), but also for SCLC significant differences were found. One explanation for this finding, as indicated by the authors, could be provided by incomplete staging procedures. In bronchial carcinoma complete staging often requires surgical procedures which physicians may tend to avoid in elderly. However, if such a relationship in fact exists, it is of particular interest because extent of disease of SCLC is strongly predictive for survival: patients who present with ED have less chance to achieve CR on chemotherapy (48, 49) and consequently will have a decreased MST compared to patients with LD.

One might expect the probability to present with a lower tumor burden to be a favourable factor towards a better prognosis. However, a review of SWOG data (50) showed a statistically significant higher frequency of CR in patients with LD who were < 55 years of age. Survival was not analyzed by age. This finding was confirmed by the study of Maurer (28); patients > 70 years with LD tended to have a lower CR rate. No such difference could be found for ED patients in which equal response rates were found. In contrast, Poplin et al (27) found lower CR rates in patients younger than 55 years with LD compared to patients > 65 years with LD (CR proportion 0.41 versus 0.75). For ED these figures were 59 versus 40%. Harper et al (49) found a consistent relationship between intrathoracic tumor size and response rate when analyzed according to disease extent and age. Also, in the study of Osterlind et al (42) age had no influence on probability to reach a CR in either LD or ED.

4. AGE AND SENSITIVITY TO CHEMOTHERAPY

Another aspect of biological behaviour of tumors is sensitivity to chemotherapy; it might be possible that tumors in elderly patients are not as sensitive to chemotherapy as tumors in younger patients are (or vice versa). Striking evidence has been seen for the negative effect of age on response in Hodgkin's disease (51). As stated above, for SCLC conflicting data exist. Intercurrent disease seems to have no effect on tumor responsiveness in either Hodgkin's disease (51) or SCLC (52).

Age over 70 years often is an exclusion criterium 'or entry in disease oriented phase II and III studies. Therefore, activity of commonly used cytotoxic agents or combinations of these agents are not fully delineated in elderly patients with SCLC. Only a few studies report on this subject (53-56) (Table I). The results of these studies show similar response rates in elderly patients as can be achieved in young patients, and a comparable MST, except for the study of Gatzemeier et al (56), in which only a 30% response rate was seen. However, in these studies single agents or two drug regimen have been administered, often with epipodophyllotoxin derivates. No information is available for other agents in this respect.

Table I.   Results of phase II studies in elderly patients (> 70 years) with SCLC.

Author	Ref.	Regimen	Response rate	Med.survival
Allan et al.	53	Vindesine 3 mg/m^2 VP 16-213* 120 mg/m^2 x 3	72%	8 months
Bork et al.	54	VM-26* 60 mg/m^2 x 5	90%	8+ months
Smit et al.	55	VP 16-213* 800 mg/m^2	76%	9 months
Gatzemeier	56	4-Epirubicin 25 mg/m^2 weekly	30%	15+ months (responding pts).

*VP-16 = Etoposide
 VM-26 = Teniposide

5. AGE AND TOXICITY OF CHEMOTHERAPY

5.1. Theoretical considerations

Concerns about increased toxicity of chemotherapy in elderly patients may in part explain why physicians withhold this form of therapy from them. Apart from physical, psychological and socio-economic factors which often interfere with the ability to obtain and to comply with health care, increased toxicity of cytotoxic agents may be due to age related changes in pharmacokinetics and -dynamics. Especially for drugs with a narrow therapeutic index such as cytotoxic agents these changes may easily lead to enhancement of side effects. In the recent years several authors have addressed this subject (57-62), and some aspects will be outlined below.

A. Absorption may be altered by physiological changes in old age, such as increased gastric pH and decreased splanchnic blood flow. No information is available with regard to this item as related to cytotoxic drugs.

B. Volume of distribution is very likely to be altered as a relative increase of body fat occurs with ageing (63). In this situation, lipophilic drugs will have an increased volume of distribution, whereas it may become smaller for hydrophylic drugs. Together with a

decreased clearance the latter may in part explain why a hydrophilic drug such as doxo-rubicin produces more cardiotoxicity in some elderly patients (64-66).

C. Metabolism of drugs occurs primarily in the liver. This complex process can be divided in oxidative and conjugative transformation. These two enzymatic pathways are not uniformly affected by ageing (58, 67); oxidation decreases with age, obviously more in men than in women, whereas for conjugative processes no changes with ageing are seen. Also, hepatic mass and hepatic blood flow decline up to 40% with age.

D. Clearance by the kidney is a function of renal blood flow, glomerular filtration and tubular secretion. All these parameters decrease with ageing; glomerular filtration rate declines about 35% between ages 20 and 90 years (68). This accounts for the most defini-tely proven pharmacokinetic deficiency in the elderly. Accordingly, drugs that depend pri-marily on clearance by the kidney must be handled with caution. The increased ototoxi-city of cisplatin, bone marrow toxicity of methotrexate and pulmonary toxicity of bleo-mycin in some elderly (69) can be explained by decreased renal function.

E. End-organ sensitivity. Although not supported by experimental data, elderly patients are believed to respond more pronounced to some drugs as younger adults. Pharmacokinetic changes as mentioned above, can only partly explain these differences (70) suggesting that elderly patients are more sensitive to the pharmacologic action of the drug. No information is available on the effect, if any, of age on end organ sensitivity to chemotherapeutic agents.

F. Although there is still controversy on whether or not effects of co-medication and drug-drug interactions occur more frequently in the elderly, clearly they are more at risk because of the larger number of drugs they take (71). Drug-drug interactions may occur in elderly patients with cancer, resulting in either augmentation or decrease of pharmaco-logic activity. Some of these interactions of commonly used drugs in SCLC are listed in Table II (72-82). It should be noted that none of these interactions definitely have been proven.

The above mentioned changes in pharmacodynamics and kinetics may result in an altered area under the plasma concentration versus time curve (AUC), as this parameter is defined by dose/total body clearance. It reflects the total exposure of the body to a given drug and is therefore the major determinant of biological effects of cytotoxic drugs. There have been no direct studies on the effect of age on the pharmacokinetics of either orally or parenterally administered cytotoxic drugs. However, one might speculate that as total body clearance is very likely to be reduced in elderly persons the net result will be a higher AUC, hence higher toxicity (61). An additional contributing factor to enhanced drug toxi-city may be reduced organ reserve. Although the capacity of the bone marrow to regene-rate following cytotoxic therapy is retained with ageing (21), the declining bone marrow cellularity (83) renders the old patient less tolerant for a given drug dose (84). B-lympho-cyte and T-lymphocyte function both decrease with advanced age, maybe making elderly patients more susceptible to septic complications of myelosuppressive chemotherapy as suggested by Armitage et al (85). Mucosal injury of the gastro-intestinal tract and its per-sistence could lead to increased endogenous infectious morbidity and mortality in older

patients, resulting from a variety of factors, including less effective repair mechanisms (86).

Table II. Drug-drug interactions of some commonly used cytostatic agents in SCLC.

Drug	Interacting drug(s)	Effects	Ref.
Doxorubicin	Barbiturates	Increased plasma clearance of doxorubicin	(72)
	Digoxin	Decreased gastrointestinal absorption	(73)
	Cyclophosphamide	Decreased plasma clearance of doxorubicin	(74)
		Alleged enhanced cardiotoxicity	(75)
	Warfarin	Excessive hypoprotrombinemia	(76)
Cyclophosphamide	Digoxin	Decreased gastrointestinal absorption	(73)
	Chlorpromazin	Increased pharmacologic action of cyclophosphamide	(77)
	Corticosteroids	Inhibition of bioactivation of cyclophosphamide	(78)
		Clinical consequences ?	(79)
	Thiazide-diuretics	Increased myelosuppression	(80)
	Cimetidine	Increased exposure to cyclo-phosphamide	(81)
Etoposide	Warfarin	Excessive hypoprotrombinemia	(82)
Vincristine	Digoxin	Reduced digoxin serum levels due to reduced gastrointestinal absorption	(73)

5.2. Clinical practice

Retrospective studies examining toxicity to chemotherapy, have generally yielded conflicting results. Begg et al (87, 88) reported on toxicity in elderly patients treated on various ECOG protocols for 7 primary sites including lung cancer. With the exception of methyl-CCNU and methotrexate, which caused a significant greater incidence of haematological toxicity, no increase in frequency or severity of toxicity in elderly patients was found. Although the authors specifically stated that no selection of elderly patients in respect to important prognostic features had occurred, one cannot exclude this possibility. The patient population was selected in terms of protocol eligibility criteria requiring normal renal, hepatic and cardiovascular function. Therefore, the sample might not be representative of the elderly population in general. Poplin et al (27) reviewed toxicity of several CDE (Cyclophosphamide, Doxorubicin and Etoposide) containing protocols and of patients not eligible for these protocols but also treated with this regimen. There were no age restrictions, thus meeting some of the above mentioned objections. Haematological toxicity was significantly enhanced in patients > 65 years. Also, hospital admissions for

neutropenia associated febrile episodes were more frequent in the older age groups, despite dose adjustments in subsequent courses. Out of 29 sudden and toxic deaths 10 occurred in patients over 65 years, as opposed to 5 in patients < 55 years. Sepsis contributed significantly to death in 16 patients. However, when death from all causes was evaluated, initial poorer performance status but not age was significant in predicting sudden/toxic death. Remarkable difference in toxicity of single agent VM-26 in elderly patients with SCLC was reported by two authors. Bork et al (54) saw virtually no toxicity in a group of 33 patients with 27 older than 70 years. Using an identical regimen Cerny et al (89) treated 30 patients with a median age of 73. Nine patients died during the first cycle of chemotherapy, five of documented sepsis. Response rates were also quite different; 90% vs. 48%. The only major difference between the two groups was the inclusion of seven patients with ECOG performance status 3 in the study of Cerny, whereas Bork excluded these patients. Apart from the latter, no satisfactory explanation for this marked difference in toxicity could be provided.

6. SUMMARY AND CONCLUSIONS

The increasing proportion of elderly persons in the population at risk, poses new problems in cancer management. Unfortunately, in the past these problems have not been studied to the extent as they have in younger patients. From the available data in the literature it is not certain whether high age is an adverse prognostic feature, especially in SCLC, either in terms of survival or tumor responsiveness. Also it is not likely, despite theoretical considerations, that all elderly patients are more prone to suffer from side effects of cytotoxic therapy. Apart from improving the therapeutic index of systemic anti-cancer therapy (90) in general, clearly more research is needed in the field of chemotherapy for elderly patients with cancer. The first step should be to systematically include elderly patients in future clinical trials in order to develop appropriate treatment regimen for them (91). Also, the precise activity and toxicity of already existing chemotherapy combinations should be evaluated in elderly persons. Next, it may be that biological rather than chronological age is more important in tolerance and response to chemotherapy (23). To date, assessment of biological age is not possible and future research should also be directed in this area.

In summary, many issues involved in chemotherapy of elderly persons with cancer remain unresolved. Until properly designed prospective clinical trials have been performed which provide answers to these questions, obviously age alone cannot be used as an isolated argument not to treat elderly patients with the same intent as in young patients.

REFERENCES

1.   Yancik R. - Frame of reference: old age as the context for the prevention and treatment of can-
        cer. In: Yancik R, Carbone PP (eds): Perspectives on prevention and treatment of cancer in
        the elderly. New York, Raven Press, 1983, pp 5-17.
2.   Centraal Bureau voor de Statistiek (CBS). - Bevolkingsprognose voor Nederland 1985-2030.
        Den Haag, Staatsuitgeverij, 1985.
3.   Horm JW, Kessler LG. - Falling rates of lung cancer in men in the United States. Lancet,
        1986, i, 425-426.
4.   Comprehensive Cancer Center South - Samenwerkingsorgaan Oncologie Ziekenhuizen
        (IKZ-SOOZ). Cancer incidence in The Netherlands: South Eastern part 1978-1982.
        Eindhoven: SOOZ Cancer Registration Office, 1985.
5.   Baranowsky A, Myers MH. - Cancer incidence and survival in patients 65 years of age and
        older. CA Cancer J Clin, 1986, 36, 26-41.
6.   O'Rourke MA, Feussner JR, Feigl P, Laszo J. - Age trends of lung cancer stage at diagnosis.
        JAMA, 1987, 258, 921-926.
7.   Stjernswärd J, Stanley K. - Lung cancer - A world wide health problem. Lung Cancer, 1988,
        4 suppl., Pl1-P14.
8.   Parkin DM, Whelan S. - Trends of lung cancer world wide. Lung Cancer, 1988, 4 suppl., P15.
9.   Ianuzzi MC, Scoggin CH. - Small cell Lung Cancer, state of the art. Am Rev Resp Dis, 1986,
        134, 593-608.
10.   Teeter SM, Holmes FF, McFarlane MJ. - Lung carcinoma in the elderly population, influence
        of histology on the inverse relationship of stage to age. Cancer, 1987, 60, 1331-1336.
11.   Kreyberg C. - Aetiology of lung cancer. A morphological, epidemiological and experimental
        analysis. Oslo, Universitets forlaget, 1969.
12.   Augusseau S, Fior M, Malka J, Mouriquand J, Seroze N. - Small cell epithelioma in the
        Rhone-Alp region: changes in incidence between 1970 and 1980. Oncology overview, selec-
        ted abstracts on small cell lung cancer, abstract no.3, 1, April 1986.
13.   Butler C, Samet JM, Humble CG, Sweeny ES. - Histopathology of lung cancer in New
        Mexico, 1970-72 and 1980-82. J Natl Cancer Inst, 1987, 78, 85-90.
14.   Tanaka I, Matsubara O, Kasuga T, Takemura T, Inoue M. - Increasing incidence and changing
        histopathology of primary lung cancer in Japan. A review of 282 autopsied cases. Cancer,
        1988, 62, 1035-1039.
15.   Oldham RK, Greco FA. - Small Cell Lung Cancer: a curable disease. Cancer Chemother
        Pharmacol, 1980, 4, 173-177.
16.   Editorial. Small cell carcinoma of the bronchus. - Real progress is hard to come by. Lancet,
        1980, i, 77-78.
17.   Goodwin JS, Hunt WC, Humble MS, Key CR, Samet JM. - Cancer treatment protocols - Who
        gets chosen. Arch Int Med, 1988, 148, 2258-2260.
18.   Samet JM, Hunt WC, Key C, Humble CG, Goodwin JS. - Choice of cancer therapy varies
        with age of patient. JAMA, 1986, 225, 3385-3390.
19.   Greenberg ER, Chute CG, Stukel T, Baron JA, Freeman DH, Yates J, Korson R. - Social and
        economic factors in the choice of lung cancer treatment. A population based study in two rur-
        al states. N Engl J Med, 1988, 318, 612-617.
20.   Begg CB, Zelen M, Carbone PP, McFadde ET, Brodovsky H, Engstrom P, Hatfield A, Ingle
        J, Schwartz B, Stolbach L. - Cooperative groups and community hospitals. Measurement of
        impact in the community hospitals. Cancer, 1983, 52, 1760-1767.
21.   Longo UL, Carbone PP. - Chemotherapy administration to the elderly patient. In: Clinics in
        Oncology, 1985, Vol 4. no 3, pp.473-484.

22.   Peterson BA, Kennedy BJ. - Aging and cancer managment. Part I: clinical observations. CA, 1979, 29, 322-332.

23.   Neilan BA. - The effect of age on prognosis. In: Lipschitz DA., moderator: Cancer in the elderly; basic science and clinical aspects. Ann Int Med, 1985, 102, 225-226.

34.   Rossing TH, Rossing RG. - Survival in lung cancer. An analysis of the effects of age, sex, resectability and histopathologic type. Am Rev Resp Dis, 1982, 126, 771-777.

25.   Lanzotti VJ, Thomas DR, Boyle LE, Smith TE, Gehan EA, Samuels ML. - Survival with inoperable lung cancer. An integration of prognostic variables based on simple clinical crite-ria. Cancer, 1977, 39, 303-313.

26.   Coy P, Elwood JM, Coldman AJ. - Clinical indicators of prognosis in unresected lung cancer. Chest, 1981, 80, 453-458.

27.   Poplin E, Thompson B, Whitacre M, Aisner J. - Small cell carcinoma of the lung: influence of age on treatment outcome. Cancer Treat Rep, 1987, 71, 291-296.

28.   Maurer LH, Pajak TF. - Prognostic factors in small cell lung cancer: A Cancer and Leukemia Group B study. Cancer Treat Rep, 1981, 65, 767-774.

29.   Osterlind K, Andersen PK. - Prognostic factors in small cell lung cancer: multivariate model based on 778 patients treated with chemotherapy with or without irradiation. Cancer Res, 1986, 46, 4189-4194.

30.   Hansen HH, Dombernowsky P, Hirsch FR. - Staging procedures and prognostic features in small cell anaplastic bronchogenic carcinoma. Sem Oncol, 1978, 5, 280-287.

31.   Feld R, Evans WK, Coy P, Hodson J, MacDonald AS, Osoba D, Payne D, Shelley W, Pater JL. - Canadian multicenter randomized trial comparing sequential and alternating administ-ration of two non cross resistant chemotherapy combinations in patients with limited small cell carcinoma of the lung. J Clin Oncol, 1987, 5, 1401-1409.

32.   Ihde DC, Makuch RW, Carney DN, Bunn PA, Cohen MH, Matthews J, Minna JD. - Prognostic implications of stage of disease and sites of metastases in patients with small cell carcinoma of the lung treated with intensive combination chemotherapy. Am Rev Resp Dis, 1981, 123, 500-507.

33.   Edmonson JH, Lagakos SW, Selawry OS, Perlia CP, Bennet JM, Muggia FM, Wampler G. - Cyclophosphamide and CCNU in the treatment of inoperable small cell carcinoma and ade-nocarcinoma of the lung. Cancer Treat Rep, 1976, 60, 925-932.

34.   Vincent MD, Ashley SE, Smith IE. - Prognostic factors in small cell lung cancer: a simple prognostic index is better than conventional staging. Eur J Cancer Clin Oncol, 1987, 23, 1589-1599.

35.   Souhami RL, Bradbury I, Geddes DM, Spiro SG, Harper PG, Tobias JS. - Prognostic signifi-cance of laboratory parameters measured at diagnosis in small cell carcinoma of the lung. Cancer Res, 1985, 45, 2878-2882.

36.   Feld R, Deboer G, Evans WK. - Prognostic factors in patients with small cell carcinoma of the lung. Proc Am Assoc Cancer Res, 1983, 24, 575.

37.   Kelly P, O'Brien A, Daly P, Claney L. - Small cell lung carcinoma in the elderly: the case for chemotherapy. Proc Brit Thor Soc Thorax, 1988, 43, 224P-225P.

38.   Smith IE, Sappino AP, Bondy PK, Gilby ED. - Long term survival five years or more after combination chemotherapy and radiotherapy for small cell lung carcinoma. Eur J Cancer Clin Oncol, 1981, 17, 1249-1253.

39.   Johnson BE, Ihde DC, Bunn PA, Becker B, Walsh T, Weinstein ZR, Matthews MJ, Whang-Peng J, Makuch RW, Johnston-Early A, Lichter AS, Carney DN, Glatstein E, Minna JD. - Patients with small cell lung cancer treated with combination chemotherapy with or without irradiation. Ann Int Med, 1985, 103, 430-438.

40.   Niiranen A. - Long term survival in small cell carcinoma of the lung. Eur J Cancer Clin Oncol, 1988, 24, 749-752.

41. Osterlind K, Hansen HH, Hansen M, Dombernowsky P, Andersen PK. - Long term disease free survival in small cell carcinoma of the lung. A study of clinical determinants. J Clin Oncol, 1986, 4, 1307-1313.
42. Osterlind K, Hansen HH, Dombernowsky P, Hansen M, Andersen PK. - Determinants of complete remission induction and maintenance in chemotherapy with or without irradiation of small cell lung cancer. Cancer Res, 1987, 47, 2733-2736.
43. Sierocki JSW, Hilaris BS, Hopfan S, Golbey RB, Wittes RE. - Small cell carcinoma of the lung, experience with a six - drug regimen. Cancer, 1980, 45, 417-422.
44. Stanley KE. - Prognostic factors in lung cancer. In: Aisner J (ed) Contemporary issues in clinical oncology no. 3: Lung Cancer. New York, Churchill Livingstone Inc., 1985, pp 45-54.
45. Cohen HJ, Bartolucci A. - Age and the treatment of multiple myeloma. Southeastern Study Group experience. Am J Med, 1985, 79, 316-324.
46. Holmes FF, Hearne E. - Cancer stage - to - age relationship. Implications for cancer screening in the elderly. J Am Geriatr Soc, 1981, 29, 55-57.
47. Goodwin JS, Samet JM, Key CR, Huble C, Kutvirt D, Hunt C. - Stage at diagnosis of cancer varies with the age of the patient. J Am Geriatr Soc, 1986, 34, 20-26.
48. Ihde DC, Makuch RW, Carney DN, Bunn PA, Cohen MH, Matthews J, Minna JD. - Prognostic implications of stage of disease and sites of metastases in patients with small cell carcinoma of the lung treated with intensive combination chemotherapy. Am Rev Resp Dis, 1981, 123, 500-507.
49. Harper PG, Souhami RL, Spiro SG, Geddes DM, Guimares M, Fearon F, Smyth JF. - Tumor size, response rate and prognosis in small cell carcinoma of the bronchus treated by combination chemotherapy. Cancer Treat Rep, 1982, 66, 463-470.
50. Reed RC, Livingston RB. - Prognostic significance of pretreatment variables in small cell carcinoma of the lung. Proc Am Assoc Cancer Res and ASCO, 1977, 18, 307.
51. Peterson BA, Pajak TF, Cooper MR, Nissen NI, Glidewell OJ, Holland JF, Bloomfield CD, Gottlieb AJ. - Effect of age on therapeutic response and survival in advanced Hodgkins disease. Cancer Treat Rep, 1982, 66, 889-898.
52. Clamon GH, Ander HW, Purnick S. - Small cell lung carcinoma in the elderly. J Am Geriatr Soc, 1982, 30, 299-302.
53. Allan SG, Gregor A, Cornbleet MA, Leonard RCF, Smyth JF, Grant IWB, Crompton GR. - Phase II trial of vindesin and VP 16-213 in the palliation of poor prognosis patients and elderly patients with small cell lung cancer. Cancer Chemother Pharmacol, 1984, 13, 106-108.
54. Bork E, Hansen M, Dombernowsky P, Hansen SW, Pedersen AG, Hansen HH. - Teniposide (VM-26), an overlooked highly active agent in small cell lung cancer. Results of a phase II trial in untreated patients. J Clin Oncol, 1986, 4, 524-527.
55. Smit EF, Carney DN, Harford P, Sleijfer DTh, Postmus PE. - A phase II study of oral etoposide in elderly patients with small cell lung cancer. Submitted for publication.
56. Gatzemeier U, Heckmayr M. - Mono-chemotherapy of small cell lung cancer with a weekly regimen of 4-epirubicin in elderly patients. Proc ECCO, 1987, 4, 24.
57. Vestal RE. - Drug use in the elderly: a review of problems and special considerations. Drugs, 1978, 16, 358-382.
58. Ouslander JG. - Drug therapy in the elderly. Ann Int Med, 1981, 95, 711-722.
59. Bressler R. - Drug use in elderly patients. In: Lipschitz DA, moderator. Cancer in the elderly: basic science and clinical aspects. Ann Int Med, 1985, 102, pp 223-225.
60. Greenblatt DJ, Abernethy DR, Shader RI. - Pharmacokinetic aspects of drug therapy in the elderly. Ther Drug Monitoring, 1986, 8, 249-255.
61. Dodion P. - Chemotherapy in the elderly. Eur J Cancer Clin Oncol, 1987, 23, 1833-1835.
62. Swift CG. - Prescribing in old age. Br Med J, 1988, 296, 913-915.

63. Forbes GB, Reina JC. - Adult lean body mass declines with age: some longitudinal observations. Metabolism, 1970, 19, 653-655.

64. Robert J, Hoerni B. - Age dependence of early phase pharmacokinetics of doxorubicin. Cancer Res, 1983, 43, 4467-4469.

65. Von Hoff DD, Layard MW, Basa P, Davis HL, von Hoff AL, Rozencweig M, Muggia FM. - Risk factors for doxorubicin-induced congestive heart failure. Ann Int Med, 1979, 91, 710-717.

66. Dresdale A, Bonow RO, Wesley R, Palmeri ST, Barr L, Mathison D, D'Angelo T, Rosenberg SA. - Prospective evaluation of doxorubicin induced cardiomyopathy resulting from postsurgical adjuvant treatment of patients with soft tissue sarcomas. Cancer, 1983, 52, 51-60.

67. Greenblatt DJ, Sellers EM, Shader RI. - Drug disposition in old age. N Engl J Med, 1982, 306, 1081-1088.

68. Friedman SA, Raizner AE, Rosen H, Salomon MA, Sy W. - Functional defects in the ageing kidney. Ann Int Med, 1972, 76, 41-45.

69. Wynne H, Woodhouse KW. - The disposition of cytotoxic drugs in the elderly. In: Hamblin TJ (ed). Clinical haematology, vol.1, no.2: Haematological problems in the elderly. Baillière Tindall, London, 1987, pp.513-531.

70. Bender AD. - Drug sensitivity in the elderly. In: Crooks J, Stevenson JH (eds). Drugs and the elderly. Perspectives in geriatric clinical pharmacology. The Mae Millan Press Ltd., London 1979, pp.147-153.

71. Lamy PP. - The elderly and drug interactions. J Am Geriatr Soc, 1986, 34, 586-592.

72. Riggs CE, Engel S, Wesley M, Wiernik PH, Bachur NR. - Doxorubicin pharmacokinetics: prochlorperazine and barbiturate effects. Clin Pharmacol Ther, 1982, 31, 263.

73. Kuhlmann J, ZIlly W, Wilke J. - Effects of cytostatic drugs on plasma level and renal excretion of ß-acetyldigoxin. Clin Pharmacol Ther, 1981, 30, 518-527.

74. Riggs CE, Akman SR, Angelou JE, Van Echo DA, Whitacre M, Bachur NR. - Doxorubicin metabolism is altered by combination therapy with cyclophosphamide and whole body hyperthermia. Proc Am Assoc Cancer Res, 1982, 1, 26.

75. Minow RA, Benjamin RS, Lee ET, Gottlieb JA. - Adriamycin cardiomyopathy-risk factors. Cancer, 1977, 39, 1397-1402.

76. Seiffer EJ, Brooks BJ, Urba WJ. - Possible interactions between warfarin and antineoplastic drugs. Cancer Treat Rep, 1985, 69, 244-245.

77. Maguire A. - Treatment of mycosis fungoides with cyclophosphamide and chlorpromazine. Br J Derm, 1968, 80, 54-57.

78. Faber OK, Mouridsen HT, Skovsted L. - The biotransformation of cyclophosphamide in man: influence of prednisone. Acta Pharmacol Toxicol, 1974, 35, 195-200.

79. Mouridsen HT, Witten J, Frederiksen PL, Hulsbach I. - Studies on the correlation between rate of biotransformation and haematological toxicity of cyclophosphamide. Acta Pharmacol Toxicol, 1978, 43, 328-330.

80. Orr LE. - Potentiation of myelosuppression from cancer chemotherapy and thiazide diuretics. Drug Intell Clin Pharm, 1981, 15, 967-970.

81. Anthony LB, Long QC, Struck RF, Hande KR. - Cimetidine increases cyclophosphamide metabolic exposure. Proc Am Assoc Cancer Res, 1988, 29, 263.

82. Ward K, Bitran JD. - Warfarin, etoposide and vindesine interactions. Cancer Treat Rep, 1984, 68, 817-818.

83. Kruger A. - The limits of normality in elderly patients. In: Hanblin TJ (ed). Clinical haematology, vol. 1, no.2: Haematological problems in the elderly. Baillière Tindall, London, 1987, pp.271-289.

84. Hoagland HC. - Hematologic complications of cancer chemotherapy. Sem Oncol, 1982, 9, 95-102.

85. Armitage JO, Potter JF. - Aggressive chemotherapy for diffuse histiocytic lymphoma in the elderly. Increased complications with advancing age. J Am Geriatr Soc, 1984, 32, 269-273.

86. Kahn SB, Begg CD, Mazza JJ, Bennet JM, Bonner H, Glick JH. - Full dose versus attenuated dose daunorubicin, cytosin arabinoside and 6-thioguanine in the treatment of acute non lymphocytic leukemia in the elderly. J Clin Oncol, 1984, 2, 865-870.

87. Begg CB, Cohen JL, Ellerton J. - Are the elderly predisposed to toxicity from cancer chemotherapy. An investigation using data from the eastern cooperative oncology group. Cancer Clin Trials, 1980, 3, 369-374.

88. Begg CB, Carbone PP. - Clinical trials and drug toxicity in the elderly. The experience of the eatern cooperative oncology group. Cancer, 1983, 52, 1986-1992.

89. Cerny T, Pedrazzini A, Joss RA, Brunner KW. - Unexpected high toxicity in a phase II study of teniposide (VM-26) in elderly patients with unresected small cell lung cancer. Eur J Cancer Clin Oncol, 1988, 24, 1791-1794.

90. Carter SK. - Improving the therapeutic index of systemic therapy with special emphasis on cisplatin and carboplatin. Cancer Treat Rev, 1987, 14, 179-185.

91. Butler RN, Gastel B. - Ageing and cancer management. Part II: Research perspectives. CA, 1979, 29, 333-340.

A PHASE II STUDY OF ORAL ETOPOSIDE IN ELDERLY PATIENTS WITH SMALL CELL LUNG CANCER

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SUMMARY

Thirty-five previously untreated patients with small cell lung cancer older than 70 years were treated with oral etoposide (800 mg/m² over 5 consecutive days) every 4 weeks. Twenty-two patients had extensive disease and 13 limited disease. The overall response rate was 71%. The median survival for patients with limited diseases was 16 (range 6-32) months and for patients with extensive disease nine (range 4-17) months. There was mild haematological toxicity and alopecia but no major toxicity. It is concluded that etoposide in this dose regimen is an effective and well tolerated treatment for elderly patients with small cell lung cancer.

INTRODUCTION

For most patients with small cell lung cancer the introduction of combination chemotherapy has considerably prolonged median survival (1) in addition to improving the quality of life. For elderly patients, however, aggressive combination chemotherapy is often associated with life threatening toxicity, especially myelosuppression. For these reasons elderly patients often have been and still are excluded from most clinical phase II trials. Treatment of these patients, however, is often indicated.

One of the most active agents for small cell lung cancer is the epipodophyllotoxin derivate etoposide (VP 16-213), which has been associated with response rates of up to 65% when used as a single agent in previously untreated patients (3). It is associated with relatively mild toxicity, mainly myelosuppression. Etoposide has clearcut dose and schedule related activity; a five day regimen has been shown to be superior to a single infusion of the same total dose in patients with small cell lung cancer (4). Etoposide is available for oral administration, after which bioavailability is about half, though there is considerable interpatient and intrapatient variability (5). We have evaluated the activity and toxicity of orally administered etoposide as palliative treatment in elderly patients with small cell lung cancer.

METHODS

Thirty five consecutive patients (33 of them male) with previously untreated small cell lung cancer were treated from August 1985 to October 1987 with the same protocol (for their characteristics see table I). All met the following criteria: cytologically or histologically proven small cell lung cancer, age over 70 years, ECOG performance score 0-3. The extent of their disease was assessed on the basis of findings at physical examination, biochemical profile and chest radiograph. If there was clinical suspicion or biochemical or radiological evidence of further extension, isotope bone scanning or liver ultrasonography or both were performed. Limited disease was defined as evidence of disease within one hemithorax and supraclavicular fossa and extensive disease as evidence of disease beyond these borders. Twenty-two patients had extensive disease and 13 limited disease.

Table I.   Patient characteristics.

	Number of patients
male : female	33 : 2
Age median (range)	73 (70-95) years
ECOG[1] performance score	
0	2
1	10
2	10
3	13
Extent of disease	
Limited (LD)	13
Extensive (ED)	22
Response rate(%)	71
Remissions:	
Complete	5 LD, 1 ED
Partial	5 LD, 14 ED
Stable disease	3 LD, 5 ED
early deaths	2 ED
Survival (median (range) mo):	
LD	16 (6-32)
ED	9 (4-17)

[1]ECOG-Eastern Cooperative Oncology Group.

Each cycle of etoposide consisted of a total dose of 800 mg/m2 body surface area, divided over five consecutive days. Etoposide was given orally, in 50 or 100 mg capsules, (Vepesid®, Bristol Myers) supplied by the patient's pharmacist or general practitioner, individual doses being rounded up or down to the nearest 50 or 100 mg. Treatment was repeated every four weeks on an outpatient basis. Chemotherapy was discontinued if there were signs of progressive disease; patients who responded received a maximum of 12 courses.

Toxicity and response were scored according to WHO criteria (6) three weeks after each treatment. This was usually the only hospital visit during the treatment cycle. Patients were considered evaluable if they had completed at least one treatment cycle. Response was assessed by physical examination and chest radiography. Bronchoscopy was not repeated. A complete remission was defined as complete regression of evaluable tumour, and a partial response as a decrease of 50% of the product of two perpendicular diameters of measurable lesions or a 30% decrease of one diameter of evaluable lesions. Adjustment of the dose to 75% of the previous dose was carried out if full haematological recovery had not occurred three weeks after the previous cycle.Survival time was determined from the start of treatment.

RESULTS

The total number of cycles was 205 and the median number of cycles given was six (range 1-12).

Response and survival
Thirty-three patients were evaluable for their response (table I); two patients died during the first cycle owing to progression of the tumor. The overall response rate was 71%, six patients showing complete regression and 19 partial regression (five limited disease, 14 extensive disease). Median survival was 16 months (range 6 - 32 months) for the limited disease group of patients and nine months (range 4 - 17 months) for those with extensive disease. One patient is still alive 22 months after the start of treatment.

Toxicity
As expected, bone marrow suppression was the predominant form of toxicity, though the incidence was low. There were no hospital admissions for drug related toxicity, including neutropenia, thrombocytopenia or anaemia. Only one patient needed adjustment of the dose and no deaths were related to treatment.

All patients experienced alopecia, usually complete. Gastrointestinal toxicity was easy to handle. Only a few patients needed symptomatic treatment.

DISCUSSION

The proportion of patients with small cell lung cancer who are over 70 years is not clear. In a report by Kreyborg in 1969 (7) only 4% of the patients with small cell lung cancer were over 70, though in a recent large American survey 26% were over 70 (8). In our institutions about 15% of patients with small cell lung cancer are older than 70. Concerns about increased toxicity of combination chemotherapy in elderly patients may be the reason why physicians tend to withold this approach (9).

Various reports are available on palliative treatment of elderly patients or those with a poor prognosis (in virtue of their performance score or extent of disease). In two studies (10,11) using two drug regimen including etoposide response rates were around 70%. The results for our patients with extensive disease - a 70% response rate and a median survival of nine months - are in agreement with the results of these studies. For the patients with limited disease the median survival was somewhat longer than in the study of Allan et al (10); 16 vs. 12.5 months. We had less toxicity and no drug related deaths, and apart from alopecia no important non-haematological side effects. In these two studies the other drug in the combination, vindesine or vincristine, probably contributed to the observed toxicity. A major advantage of our treatment is that it may be given on an outpatient basis with minimal investigations and hospital visits.

The median survival for the patients in this study is very reasonable both for those with extensive disease (nine months) and for those with limited disease (16 months). Though some series report better survival for the latter group, this might be only at the cost of more treatment related toxicity. Certainly for elderly patients, who may be less able to tolerate or survive standard chemotherapy regimen, a shorter median survival with the alleviation of symptoms may be considered as an acceptable goal.

We conclude from our data that orally administered etoposide at a dose of 800 mg/m^2 divided over five consecutive days is a well tolerated and effective regimen for palliation in elderly patients with small cell lung cancer.

REFERENCES

1   Ihde DC. Current status of therapy for small cell carcinoma of the lung. Cancer 1985; 54: 2722-2728.
2   Poplin E, Thompson B, Whitacre M, Aisner J. Small cell carcinoma of the lung: influence of age on treatment outcome. Cancer Treat. Rep.1987; 71: 291-296
3   Cavalli F, Goldhirsch A, Joss R. Single-agent activity and European experiences with etoposide in the treatment of small cell lung cancer. In: Etoposide (VP16) current status and new developments, Issel BF, Muggia FM, Carter SK (eds.) - Academic Press, Orlando Florida 1984, p. 163-169.
4   Slevin ML, Clark PI, Osborne RJ, Malik S, Wood CD, Harvey VJ, Malpas JS, Wrigley PFM. A randomised trial to evaluate the effect of schedule on the activity of etoposide in small cell lung cancer. Proc. ASCO 1986 (abstr).
5   Stewart DJ, Hundy D, Maroun JA, Tetreault L, Prior J. Bioavailability, pharmacokinetics and clinical effects of an oral preparation of etoposide. Cancer Treat Rep 1985; 69: 269-273.
6   WHO Handbook for reporting results of cancer treatment. WHO offset publication no 48, Nyhoff, Den Haag, 1979.
7   Kreyborg C: Aetiology of lung cancer: A Morphological Epidemiological and experimental Analysis. Universitatsforlaget, Oslo, Norway 1969.
8   Teeter SM, Holmes FF, McFarlane MJ. Lung carcinoma in the elderly population, influence of histology on the inverse relationship of stage to age. Cancer 1987; 60: 1331-1336
9   Samet JM, Hunt WC, Key CR, Humble CG, Goodwin JS. Choice of cancer therapy varies with age of patient. JAMA. 1986; 225: 3385-3390.
10   Allan SG, Gregor A, Cornbleet MA, Leonard CF, Smyth JF, Grant JWB, Crompton GK. Phase II trial of vindesine and VP16-213 in the palliation of poor prognosis patients and elderly patients with small cell lung cancer. Cancer Chemother. Pharmacol. 1984; 13: 106-108.
11   Morgan DAL, Gilson D, Fletcher J. Vincristin and etoposide: an effective chemotherapy regimen with reduced toxicity in extensive small cell lung cancer. Eur J Clin Oncol 1987; 23: 619-621.

Chapter 3

PHASE I STUDY OF ORAL TENIPOSIDE

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INTRODUCTION

Podophyllotoxins have been used therapeutically for over a thousand years. One of the first reports of their application in cancer treatment stems from an early medieval English book 900 to 950 AD. Prohibitive toxicity in phase I cancer trials in the early 1950's (1) prevented their intravenous (IV) use. Not until 1967 was the first of two promising semi-synthetic podophyllotoxins, 4'demethyl-epipodophyllotoxin-9 (4,6-0-thenylidene-B-D-glucopyranoside), or VM-26 (teniposide) introduced in anticancer chemotherapy. Although it became available earlier, teniposide is somewhat of a poor relation compared with its congener etoposide regarding the extent to which its pharmacology and clinical use have been studied (2). While etoposide has been extensively studied in a wide range of haematologic and solid malignancies (3), the clinical use of teniposide has been restricted mainly to pediatric oncology, non-Hodgkin's lymphoma and brain tumors in adults (4). In the last few years interest in teniposide has been renewed which may be due to an accumulation of *in vitro* and *in vivo* data. Both etoposide and teniposide exert their cyto-toxic action by a reversible interaction with DNA-topoisomerase II, resulting in increased DNA scission and inhibition of rejoining (5,6). Recent *in vitro* work has suggested that teniposide is more potent in this regard than etoposide (7), and clinical studies indicate that teniposide is a superior compound (8). The mechanism of action of both drugs explains their schedule-dependent activity, as was demonstrated with etoposide in *in vivo* studies of small cell lung cancer: prolonged administration was found to be superior to a short infusion of the same total dose (9,10). In an out-patient setting, multiple daily IV administration is difficult to handle and rather problematic for most patients. A way to overcome this inconvenience is to substitute an oral preparation for the IV drug. Such regimen have already been used with etoposide. Oral administration of teniposide would certainly facilitate wider use of this active anticancer agent. These considerations prompt-ed us to perform a phase I study of oral teniposide, which is the subject of this report.

MATERIALS AND METHODS

Between October 1987 and June 1989, 19 patients at University Hospital Groningen were entered into this study. All patients had cytologically or histologically proven malignancies for which no other generally accepted treatment was available at the time of entry. In addition, all patients met the following criteria: (1) Eastern Cooperative Oncology Group (ECOG) performance status of 3 or less; (2) expected survival of at least 2 months; (3) serum creatinine level of <130 μmol/l; (4) serum bilirubin level <35 mmol/l; (5) WBC $>3.0\times10^9$/l; (6) platelet count $>100\times10^9$/l. None of the patients had received chemotherapy or radiotherapy for at least 4 weeks before the start of teniposide treatment. The study was approved by the local Medical Ethical Committee, and oral informed consent was obtained from all patients before entry.

Patients received oral teniposide over five consecutive days. Teniposide is only available in 50 mg vials for IV use, with benzylalcohol 0.15 g; N,N-dimethyl acetamidine 0.3 g; polyethoxylated castor oil 2.5 g; maleic acid to a pH of 5.1 and absolute alcohol to 5 ml (11). This solution was dissolved by the patients themselves in 100 ml of syrup or orange juice. To assure proper drug handling, patients were trained by an oncology nurse. The patients took the whole dose each morning before breakfast. The starting dose was established as 60 mg/m^2/day because an earlier study of teniposide 30 mg/m^2/day IV for five consecutive days produced no major side effects (12). At an expected 50% absorption level, which is similar to that of oral etoposide, 60 mg/m^2 given orally is roughly equivalent to 30 mg/m^2 IV. Courses were repeated every three weeks for a maximum of twelve courses if no disease progression was evident and no unacceptable toxicity occurred. The dose was to be escalated with 25 mg/m^2/day if no unacceptable toxicity occurred in any of at least three patients at that level during the first course. The tenipos-

Table I.   Patient characteristics.

	number of patients
Median age (range)	59 (21-74) years
Sex	
Male	17
Female	2
ECOG performance status	
0	2
1	5
2	6
3	6
Primary site	
Non small cell lung cancer	15
Adenocarcinoma of unknown origin	2
Non seminoma testis tumor	1
Liposarcoma	1
Previous therapy	
Chemotherapy (C)	3
Radiotherapy (R)	7
C + R	4
Oral VM26 dose (no courses)	
60 mg/m^2/day	3 (12)
85 mg/m^2/day	4 (22)
110 mg/m^2/day	3 (17)
135 mg/m^2/day	3 (8)
160 mg/m^2/day	6 (18)

ide dose was not escalated in the individual patient. Toxicity was evaluated on days 7, 14 and 21 of each course with a patient interview, physical examination, full blood counts and assessment of renal and hepatic function. A chest X-ray was performed every three weeks. Unacceptable toxicity, using the World Health Organisation (WHO) grading system (13), was defined as grade 3 or 4 oral mucositis, grade 3 diarrhea or grade 3 or 4 haematologic toxicity.

To be evaluable for response a patient with measurable tumor manifestations had to be followed for at least 6 weeks from the start of treatment. Complete response was defined as complete disappearance of all known tumor for at least 4 weeks and a partial response as a decrease of at least 50% in the sum of the products of the largest perpendicular diameters of all measurable lesions. Stable disease was defined as a situation with less than 50% decrease or less than 25% increase and progressive disease as more than 25% increase in the sum of products of the aforementioned diameters.

Table I lists the clinical characteristics of the nineteen patients entered in this study. For none of these patients treatment with teniposide alone is considered standard treatment. Three patients received teniposide 60 mg/m²/day, 4 patients 85 mg/m²/day, 3 patients 110 mg/m²/day, 3 patients 135 mg/m²/day and 6 patients 160 mg/m²/day. Six patients instead of three were assigned to the highest dose level because unacceptable toxicity had occurred in two of the first three patients treated with this dose.

RESULTS

Eighteen out of 19 patients completed at least one course of therapy and are evaluable for toxicity. One patient who received 160 mg/m²/day died under unknown circumstances at day 15 of the first course. Table I also lists the total number of courses administered per dose step. Nine patients received one course, one patient received two courses, one patient three courses, two patients four courses, one patient seven courses and four patients received 12 courses of oral teniposide. Reasons for discontinuing teniposide therapy was toxic death in two patients and unacceptable gastrointestinal (GI) toxicity in 3 patients. Four patients completed 12 courses and nine patients had disease progression after one course.

Toxicity
Haematological
Tables II and III show the nadirs of leukocyte and thrombocyte counts. In general, both nadirs occurred around day 14 of each course. All patients recovered sufficiently by day 21 to allow a next course to be initiated. We could not demonstrate dose-related myelosuppression, although a slight tendency towards lower median nadirs with increasing dose was evident. Instead, WHO grade 3 or 4 haematologic toxicity occurred in five patients at various dose levels. Two men treated with 85 mg/m²/day developed grade 3 leukopenia during the first course. Both patients were heavily pretreated with chemotherapy and radiotherapy and had an ECOG performance status of 3. A 41-year-old woman who had recei-

ved four courses of teniposide 110 mg/m^2/day without any sign of toxicity developed severe myelosuppression on day 11 of the fifth course. By day 13, her leukocyte count had dropped to 0.07 x 10^9/l and her platelet count to 8x 10^9/l. This patient, suffering from widely disseminated bronchial carcinoma died on day 14 due to respiratory failure. A 58-year-old man, with rapidly progressing metastatic bronchial carcinoma and an ECOG performance status of 3, died due to teniposide-induced myelosuppression. After one course of chemotherapy (4-epidoxorubicin IV) and additional radiotherapy for superior vena caval obstruction he had received one course of oral teniposide 160 mg/m^2/day. He was hospitalized because of fever on day 16, and severe myelosuppression was evident at that point. Despite appropriate antibiotic treatment he died of uncontrolled septic shock on day 21 in deep aplasia. The last patient who experienced grade 4 haematological toxicity was previously untreated, and also received 160 mg/m^2/day. He recovered without any problems on day 21. None of the remaining 13 patients experienced severe myelosuppression during the course of their treatment. Except for one patient treated with the 110 mg/m^2/day dose, there was no evidence of cumulative haematological toxicity in those patients who received more than one course of oral teniposide.

Table II. Median nadirs (range) of leukocyte counts (/μL) during 5-day course of oral VM26.

Dose	day 7	day 14	day 21
60 mg/m^2/day	6.3 (6.1- 9.4)	6.3 (4.5-9.4)	6.3 (5.6-7.1)
85 mg/m^2/day	7.8 (6.4-10.1)	5.9 (1.4-8.1)	6.0 (1.7-19.4)
110 mg/m^2/day	8.9 (7.2-11.7)	5.3 (0.03-8.5)	6.0 (4.4-15.2)
135 mg/m^2/day	6.6 (2.7-11.3)	5.4 (2.1-6.7)	4.1 (2.7-5.4)
160 mg/m^2/day	6.7 (3.4-11.7)	4.9 (0.7-9.9)	5.6 (0.03-7.2)

Table III. Median nadirs (range) of thrombocyte counts (/μL) during 5-day course of oral VM26.

Dose	day 7	day 14	day 21
60 mg/m^2/day	314 (271-389)	289 (258-508)	251 (233-269)
80 mg/m^2/day	240 (134-349)	234 (112-373)	285 (224-385)
110 mg/m^2/day	407 (302-649)	257 (9-477)	389 (310-854)
135 mg/m^2/day	305 (164-446)	215 (157-389)	346 (233-506)
160 mg/m^2/day	219 (98-374)	260 (79-472)	289 (7-445)

Non-haematological
GI toxicity was the second most common toxicity encountered. Virtually all patients complained of the unpleasant taste of the teniposide solution. Several patients retched during administration of the drug. Twelve patients received anti-emetics. Frequent vomiting persisted in 5 patients. Because of this acute toxicity two patients were withdrawn from treatment after the first course upon their request.

Grade 3 diarrhea occurred in a 74-year-old man during the first course (160 mg/m^2/day), causing discontinuation of therapy. Apart from the five patients who had severe myelosuppression, no oral mucositis was seen. No renal or hepatic toxicity occurred.

Total alopecia was not seen in the 14 patients at risk for this toxicity. Partial alopecia was frequent at dose above 85 mg/m^2/day.

No hypersensitivity reactions, such as reported by O'Dwyer et al. (14) were observed.

Response

Seven of 10 patients who received more than one course of oral teniposide are evaluable for response. No complete or partial responses were seen. All patients had stable disease with a median time to progression of 4 months (range 2-11 months). Two patients are still alive 8 and 2 months after the start of therapy with oral teniposide and treated upon the same protocol.

DISCUSSION

This is the first phase I study investigating oral teniposide administration, using a daily times 5 schedule. The maximum tolerated daily dose in this schedule was 160 mg/m^2/day. Leukocytopenia and thrombocytopenia as well as GI toxicity were dose-limiting side effects. The latter may be related to the use of the drug in a liquid form. Brunner and cow-orkers (15) saw an approximately threefold increase in GI toxicity when etoposide was administered orally as a drinking ampule compared with capsules. Also, use of a hydrophilic gelatine capsule blocked the unpleasant taste of the oral etoposide solution (16).

Myelosuppression was expected to be the dose-limiting side effect of oral teniposide. Nadirs occurred around day 14 and cell counts recovered in most patients by day 21. However, heavily pretreated patients may not be able to tolerate a cumulative oral teniposide dose of 800 mg/m^2; two such patients, who received a cumulative dose of 420 mg/m^2, had grade 3 leukopenia. In contrast, some untreated patients can probably tolerate a higher dose of oral teniposide: two patients treated at the highest dose level experienced no major myelosuppression. Apart from treatment status, interpatient and intrapatient pharmacokinetic differences in drug absorption probably also influence toxicity. Several authors report variable etoposide absorption when the drug is administered orally. Its absolute bioavailability seems to be approximately 50%, but there are large intra- and inter-individual differences; reported drug absorption has varied from less than 40% to more than 70% (16,17). The only study reporting on the absolute bioavailability of oral teniposide to date shows similar differences in absorption. Holthuis et al. (18) studied variation of bioavailability in three patients after oral drug administration over 5 days. Based on measurements of the area under the concentration versus time curve, bioavailability ranged from 19% to 57% with considerable intra-individual variation. Therefore, under-dosing or overdosing may be caused inadvertently within schedules using oral preparations of either drug, especially when the drug is administered over several days (19).

Smyth et al. (20) suggested that individual dose optimization is necessary for etoposide. However, this would require serial sampling of plasma as carried out by Stewart et al (21) who found a significant relationship between percent decrease in WBC count and clearance of non-protein-bound etoposide after IV administration. Monitoring blood concentrations for a single day after oral treatment may give a misleading idea of the total bioavailability during a course of therapy that lasts several days (22). Unfortunately, such approaches are not practical in routine use of orally administered anticancer drugs.

The absence of major responses in this group of patients is not surprising, as the majority of them suffered from non-small cell lung cancer. Teniposide is known to have only modest activity in this malignancy (23). It seems most appropriate to explore the effect of oral teniposide in a phase II setting in malignancies in which this drug appears to have a high activity, such as small cell lung cancer (7,24) or non-Hodgkin's lymphoma. Furthermore, it might be worthwhile to use oral teniposide in combination with other cytotoxic drugs. To reduce the GI toxicity, it may be preferable to supply teniposide in capsules instead of ampules. as was done for etoposide.

In conclusion, we found that the maximum tolerated daily dose of oral teniposide administered over five days is 160 mg/m^2/day. Individual dose de-escalations may be necessary in previously treated patients or in those with presumed erratic absorption of the drug, resulting in excessive myelosuppression.

REFERENCES

1. Greenspan E, Leiter J, Shear M. Effect of α-pelatin, β-pelatin and podophyllotoxin on lymphoma and other transplanted tumors. J. Nat. Cancer Inst. 10: 1295-1332, 1950.
2. Macbeth F.R. Vm26: Phase I and II studies. Cancer Chemother Pharmacol. 7:87-91, 1982.
3. O'Dwyer P.J., Leyland-Jones B., Alonso M.T., Marsoni S., Wittes R.E. Etoposide (VP16-213): current status of an active anti cancer drug. New Eng. J. Med. 312: 692-700, 1985.
4. O'Dwyer P.J., Alonso M.T., Leyland-Jones B., Marsoni S. Teniposide: A review of 12 years of experience. Cancer Treat. Rep. 68: 1455-1466, 1984.
5. Rowe T.C., Tewey K.M., Liu L.F. Identification of the breakage reunion subunit of T4 DNA topoisomerase. J. Biol. Chem. 259: 9177-9181, 1984.
6. Chen G.L., Yang L., Rowe T.C., Halligan B.D., Tewey K.M. Nonintercalative antitumor drugs interfere with the breakage reunion reaction of mammalian DNA topoisomerase II. J. Biol. Chem. 259: 9182-9187, 1984.
7. Roed H., Vindelov L.L., Christensen I.J., Spang-Thomsen M., Hansen H.H. The effect of two epipodophyllotoxin derivatives etoposide (Vp-16) and teniposide (Vm-26) on cell lines established from patients with small cell carcinoma of the lung. Cancer Chemother. Pharmacol. 19: 16-20, 1987.
8. Bork E., Hansen M., Ersbol J., Dombernowsky P., Bergmann B., Hansen H.H. A randomized study of teniposide (Vm-26) versus Vepeside (Vp-16) as single agents in previously untreated patients with small cell lung cancer (SCLC) >70 years. Proc. ASCO, 890, 1989 (abstr).
9. Cavalli F., Sonntag R.W., Jungi F., Senn H.J., Brunner K.W. Vp-16-213 monotherapy for remission induction of small cell lung cancer: a randomized trial using three dosage schedules. Cancer Treat. Rep. 62: 473-475, 1978.

10.   Clark P.I., Joel S.P., Slevin M.L. A pharmacokinetic hypothesis for the clinical efficacy of etoposide in small cell lung cancer. Proc. ASCO, 257, 1989 (abstr).

11.   Rozencweig M., Von Hoff D.D., Henney J.E., Muggia F.M. VM 26 and VP 16-213: a comparative analysis. Cancer 40: 334-342, 1977.

12.   EORTC Cooperative group for leukaemias and haematosarcomas. Clinical screening of epipodophyllotoxin VM26 in malignant lymphomas and solid tumors. Br. Med. J. 2:744-748, 1972.

13.   WHO Handbok for reporting results of cancer treatment. WHO offset publication no. 48, Nijhoff, Den Haag, 1979.

14.   O'Dwyer P.J., King S.A., Fortner C.L., Leyland-Jones B. Hypersensitivity reactions to Teniposide (VM-26): an analysis. J. Clin. Oncol. 4: 1262-1269, 1986.

15.   Brunner K.W., Sonntag R.W., Ryssel H.J., Cavalli F. Comparison of the biological activity of Vp-16-213 given iv and orally in capsules or drink ampules. Cancer Treat. Rep. 60: 1377-1379, 1976.

16.   Stewart D.J., Nundy D., Maroun J.A., Tetreault L., Prior J. Bioavailability, pharmacokinetics, and clinical effects of an oral preperation of etoposide. Cancer Treat. Rep. 69; 269-273, 1985.

17.   D'Incali M., Farina P., Sessa C., Mangioni C., Conter V., Masera G., Rocchetti M., Brambilla Pisoni M., Piazza E., Beer M., Cavalli F. Pharmacokinetics of Vp16-213 given by different administration routes. Cancer Chemother. Pharmacol. 7: 141-145, 1982.

18.   Holthuis J.J.M., Splinter T., Post M.H. Absolute bio-availability (BA) and pharmacokinetics of oral Teniposide (Teniposyrup). Proc. AACR, 873, 1988 (abstr).

19.   Clark P.I., Slevin M.L. The clinical pharmacology of Etoposide and Teniposide. Clinical Pharmacokinetics 12: 223-252, 1987.

20.   Smyth R.D., Pfeffer M., Scalzo A., Comis R.L. Bioavailability and pharmacokinetics of etoposide (Vp-16). Seminars in Oncolgy 12, suppl.2: 48-51, 1985.

21.   Stewart C.F., Arbuck S.G., Fleming R.A., Evans W.E. Leukopenia and etoposide systemic exposure: clinical relevance of nonprotein bound etoposide. Proc. ASCO, 276, 1989 (abstr).

22.   Harvey V.J., Slevin M.L., Smythe M.M., Johnston A., Wrigley P.F.M. Variable bioavailability following repeated oral doses of etoposide. Eur. J. cancer Clin. Oncol. 21: 1315-1319, 1985.

23.   Sorensen J.B. Etoposide and teniposide in non-small cell lung cancer. in: Vepesid and Vumon, a status report. Christian Christensen, Denmark, 1988.

24.   Bork E., Hansen M., Dombernowsky P., Hansen S.W., Pedersen A.G., Hansen H.H. Teniposide (Vm-26), an overlooked highly active agent in small-cell lung cancer. Results of a phase II trial in untreated patients. J. Clin. Oncol. 4: 524-527, 1986.

Chapter 4

TREATMENT OF BRAIN METASTASES OF SMALL CELL LUNG CANCER WITH TENIPOSIDE

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SUMMARY

Symptomatic brain metastases are found in 40 - 50 % of patients with small cell lung cancer. Results of whole brain radiotherapy are rather poor and recurrences after radiotherapy are frequently seen. New modes of therapy for these patients are needed. In this study the efficacy of teniposide at a dose of 150 mg/m² on day 1, 3 and 5 at 3 weeks interval is evaluated. In 11 out of 26 evaluable patients a response was seen. Median response duration was 42 weeks (range 4 - 65). Toxicity was acceptable, grade 3/4 leukocytopenia and thrombocytopenia were seen in 37% and 16% of 123 courses respectively. Teniposide is active against brain metastases of small cell lung cancer and is a suitable drug for palliation in these patients.

INTRODUCTION

Central nervous sytem (CNS) metastases are frequently seen in patients with small cell lung cancer (SCLC) (1). The vast majority of CNS metastases are brain metastases (2). They are present in about 10% of the patients at the time of diagnosis (2-4), while in 1-2% this is the sole site of documented extrathoracic disease (2). An additional 30-40% of the patients develop clinically overt brain metastases prior to death (2,5). The rate at autopsy is even higher (3) and at 2 years the probability has risen to 80% (2). The introduction of combination chemotherapy almost two decades ago has resulted in important changes in survival of patients with SCLC (6). However, for the majority of patients with CNS metastases the prognosis remains very poor. A possible explanation for this is the minimal penetration of most cytostatic drugs into the CNS. In several randomized trials the use of prophylactic cranial irradiation (PCI) has reduced the frequency of CNS metastases from about 23% to 6%, but a significant survival benefit has not been found (7).

Traditionally, treatment of clinically evident brain metastases has been whole brain radiotherapy (WBRT), usually in combination with high dose corticosteroids. Results of this treatment have only been reported in non-randomized and predominantly retrospec-

tive investigations (2,3,8,9,10). As expected, the response rates vary considerably with complete symptomatic remissions ranging from 25% to 64%. Several factors may account for the variation in response rate reported. Firstly, the possibility of incomplete neurological evaluation in retrospective studies cannot be ruled out and differences exist between the studies with respect to the degree of neurological impairment of the patients. Secondly, the number of patients with brain metastases after chemotherapy versus previously untreated patients varied in these studies. Finally, response criteria varied from one study to another.

The median survival of patients presenting with brain metastases during or after chemotherapy is apparently very short: 3 - 4.5 months (2,8-10), even with additional chemotherapy (9). However, in only a minority of the patients receiving WBRT progression of the brain metastases was the cause of death, the great majority died of tumor progression outside the brain. Regarding the poor results of WBRT for patients with brain metastases from SCLC and the lack of therapeutic possibilities for patients relapsing in the brain after either PCI or WBRT, other modes of therapy have to be evaluated.

In a pilot study of high-dose etoposide penetration into the cerebrospinal fluid was documented and also efficacy against brain metastases of SCLC was shown (11,12). However, toxicity of this regimen was too severe to consider it as a suitable regimen for palliation in these patients (13). Penetration into the cerebrospinal fluid was also found for the congener of etoposide, teniposide (14). In a patient with a relapse of brain metastases from SCLC after WBRT and high-dose etoposide, teniposide at a dose of 150 mg/m2 resulted in a response without severe toxicity (15). In this report we describe the first results of a phase II study of teniposide in patients with brain metastases of SCLC.

MATERIALS AND METHODS

Patients

From September 1987 to August 1989 twenty-nine patients were entered in this study. Criteria for eligibility in the study were: histologically or cytologically proven SCLC, leukocyte count $\geq 3.0 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, normal renal function (serum creatinine ≤ 150 mmol/l), normal bilirubin (≤ 25 mmol/l) and brain metastases documented by contrast enhanced brain computer tomography (CT). If previous therapeutic or prophylactic cranial radiotherapy had been given this had been completed more than 6 weeks before entrance into the study. Moreover, in these patients the brain metastases should be progressive, based on clinical signs and/or brain CT. Concomitant chemotherapy was not allowed. Informed consent was obtained from all patients.

Therapy

Teniposide (VM 26, Vumon®) was given at a daily dose of 150 mg/m2 on day 1, 3 and 5 at 3 weeks interval. Teniposide was diluted in 500 ml normal saline and administered IV

in 1 hour in the outpatient department. Treatment was continued till progression either in the brain or of the tumor outside the CNS. Maximally 12 courses were given. Dose reductions were not allowed for. If bone marrow recovery was not complete at the planned time for the next course treatment was delayed for 1 or 2 weeks.

Corticosteroids (dexamethasone) were given if symptoms due to edema were disabling. Two weeks after the start of therapy with teniposide the daily corticosteroid dose was to be reduced gradually to zero unless symptoms due to edema associated with brain metastases recurred. Platelet transfusions were administered when platelet counts were $\leq 10 \times 10^9/l$.

Response
Response was evaluated after each course by neurological investigation and by contrast enhanced brain CT after 2, 6 and 12 courses.

Table I.   Classification of neurological function (NF).

1	Intellectually and physically able to work; neurological findings minor or absent;
2	Intellectually intact and physically able to be at home, although nursing care may be required; neurological findings present but not a major factor;
3	Major neurological findings requiring hospitalization and medical care and supervision;
4	Requires hospitalization and is in serious physical and neurological state.

The neurological function of the patients was scored according to the classification of Order et al. (16) (table I). A complete response (CR) was defined as a complete disappearance of the tumor lesions on brain CT with a total clearing or stabilization of the neurological signs and symptoms. A partial response (PR) was defined as a decrease of 50% or more of the product of the perpendicular diameters of an enhancing lesion on the CT or a similar reduction in the sum of the products of the perpendicular diameters of enhancing lesions on CT and/or a reduction in number without otherwise signs of progression. Stable disease (SD) was defined as an increase of less than 25% or decrease of less than 50% of the enhancing lesions without development of new lesions and no worsening of neurological symptoms. Progressive disease (PD) was defined as an increase of more than 25%, or appearance of new metastases and/or neurological worsening attributed to the metastases. Toxic death (TD) is death due to treatment related toxicity and early death (ED) is death due to tumor progression before the first evaluation after the start of therapy.

The duration of response and survival were measured from the first day of the therapy with teniposide.

Toxicity
Toxicity was scored during each course according to the WHO-criteria (17). The patients were evaluated between day 14 and 16 of each course in the outpatient department.

RESULTS

Patients
Of 29 patients entered in this study there were 2 females and 27 males with a median age of 62 years (range 48 - 80). Five patients were previously untreated. Twenty-four had been treated with chemotherapy for SCLC, of these patients 20 had received etoposide at a standard dose. In 9 patients brain metastases were the only place of tumor progression, in the other 15 patients also tumor progression outside the CNS was found shortly before or at the same time as the brain metastases were found. Nine patients had received WBRT, including 2 patients with PCI. The performance score (ECOG) was 0 in 4 patients, 1 in 11 patients, 2 in 6 patients, 3 in 5 patients and 4 in 3 patients. The neurological function (NF) score of the patients was 1 in 12 patients, 2 in 11 patients, 3 in 4 patients and 4 in 2 patients. The number of metastases found on brain CT is shown in table II. Seven patients needed corticosteroids at the start of the chemotherapy.

Table II.

Number of brain metastases	number of patients
1	5
2-3	6
4-10	6
>10	12

Response
Twenty-six patients were evaluable for response, one patient died of an unknown cause at home shortly after the second course. Two patients just completed their first course and are not yet evaluated. One patient with ECOG performance score 4 died during aplasia related septicemia. Four patients are still treated according to the study protocol. Eleven patients received 1 or 2 courses, 7 patients between 3 and 6 courses and 8 patients received more than 6 courses.

Table III. Prior chemotherapy and response of brain metastases to teniposide.

	All	Untreated	Chemotherapy	
	(n=27)	(n=4)	(n=23) start of teniposide after prior chemotherapy	
Response			<3 months	>3 months
Complete	4		2	2
Partial	7	2	2	3
Stable disease	5	2	2	1
Progressive disease	9		7	2
Toxic death	1		1	
Not evaluable	1		1	

The best response scored in the patients is shown in table III.There were 4 patients with a CR, 7 patients with a PR and 5 patients had stable disease. The response rate was 42%. There is a small, but not statistically significant, difference between the group of patients relapsing in the brain after a longer treatment free interval and the patients with brain metastases becoming symptomatic during or shortly after chemotherapy. Of the patients with ECOG performance score 3-4 only 1 out of 7 responded, whereas 10 out of 19 patients with ECOG performance score 0-2 had a response. The median survival of patients with ECOG performance score 3-4 (n=8) was 8 weeks (range 4-30) and of the patients with ECOG performance score 0-2 (n=21) 21 weeks (range 4-65). Of the 6 patients with a neurological function score (NF) of 3-4 only 1 had a response, the median survival of these patients was 5.5 weeks (range 4-30). The median survival of the patients with NF 1-2 was 21 weeks (range 4-65). In the patients responding to the chemotherapy it was possible to stop the corticosteroids. Median response duration was 23 weeks (range 9 - 50). The median survival of the responding patients was 42 weeks (range 10+ - 65), whereas the nonresponders had a median survival of 12 weeks (range 4-38). All responding patients had a NF-score of 1 at the time of the response. After teniposide treatment tumor progression was in 6 patients only in the CNS and in 19 patients also outside the CNS. Of 6 patients with tumor progression in the brain 4 had a temporary improvement after WBRT.

Toxicity
Hundred and twenty-three courses have been given. In 29% of the courses grade 3 and in 8% grade 4 leukocytopenia was seen. Thrombocytopenia grade 3 was seen in 7% and grade 4 in 9% of the courses. During 4 courses (3%) platelet transfusions were given. During 7 courses (6%) fever due to infection was noted, 1 patient died during aplasia related septicemia. In all patients at risk alopecia was noted. Nausea and vomiting were mild and easily controlled by antiemetics. Grade 1 mucositis of the oropharynx was found in 1 patient.

DISCUSSION

Despite two decades of chemotherapy for SCLC the efficiency of cytotoxic drugs for preventing or treating CNS metastases has not been sufficiently studied. Adding drugs that are supposed to cross the blood-brain barrier (BBB), i.e. procarbazine, nitrosureas and high-dose methotrexate, to combination chemotherapy regimen resulted in the same frequency of CNS relapse as was seen after treatment with other cytotoxic drugs (2, 18 - 21). Also, adding standard dose etoposide to the combination regimen did not improve the CNS relapse frequency (22).

Recently in 3 small groups of patients with newly diagnosed SCLC and brain metastases, responses after standard dose combination chemotherapy have been reported (23-25). These results are remarkable and undermine the concept of the impenetrability

of the BBB. The role of this BBB might be much less important in patients with sympto-
matic brain metastases than in patients without metastases. These small studies make it
clear that it is necessary to evaluate chemotherapy for brain metastases in a more syste-
matic way. Until very recently, the evaluation of the efficacy of single agents against brain
metastases from SCLC has been done in small groups of patients only. Giaccone et al. (26)
found a response in 3 out of 8 patients with SCLC and brain metastases treated with teni-
poside at a dose of 120 mg/m^2 on days 1, 3 and 5. The 42% response rate found in the pre-
sent study confirms this result, and is comparable to the study of the EORTC Lung Cancer
Cooperative Group with high-dose etoposide in a similar group of patients (27). In 10 out
of 23 patients a response was seen (43%). Toxicity of this regimen was considerable with
5 patients who died during aplasia related septicemia and this makes this regimen unsui-
table for palliative treatment in most patients. In the present study myelosuppression was
also the most important side effect but it was less severe and only 1 patient sofar died
during aplasia.

Although teniposide is probably one of the most active drugs against SCLC (28), its
activity in patients previously treated with etoposide is minimal (29). The 42% response
rate found in this study is therefore remarkable. An explanation for this could be the mini-
mal penetration of etoposide at standard doses into the CNS (30) and the integrity of the
BBB in the situation without apparent metastases ultimately resulting in minimal or no
exposition of the metastatic tumor cells to etoposide and no induction of resistance.

Comparing this study with previously reported studies of WBRT is difficult especially
due to the much less clear response evaluation after WBRT. In the study by Lucas et al.
(10) a CR was defined as achieving and maintaining a NF score of 1 and a PR if a NF
score of 2 was reached. With respect to the NF score in the present study all patients
reached a CR, according to the definition by Lucas, which is comparable to the 46%
response rate after WBRT and corticosteroids. Survival of the responding patients in both
studies is also comparable.

The number of patients in this study is too small to draw conclusions whether it is
worthwhile to go on treating patients with brain metastases with a poor PS and/or NF sco-
re. Also the possible negative prognostic influence of recent or concurrent chemotherapy
on the chance to respond in the brain has to be evaluated in a larger group of patients.
Presently the EORTC is evaluating the same regimen in a much larger group of patients
and results of this study may throw more light on several remaining questions.

REFERENCES

1 Newman SJ, Hansen HH. Frequency, diagnosis, and treatment of brain metastases in 249 con-
   secutive patients with bronchogenic carcinoma. Cancer 1974; 33: 492-496
2 Nugent JL, Bunn PA, Matthews MJ, Ihde DC, Cohen MH, Gazdar A, Minna JD. CNS metasta-
   ses in small cell bronchogenic carcinoma. Increasing frequency and changing pattern with
   lengthening survival.Cancer 1979; 44: 1885-1893

3   Hirsch FR, Paulson OB, Hansen HH, Larsen SO. Intracranial metastases in small cell carcinoma of the lung. Prognostic aspects. Cancer 1983; 51: 529-533

4   Hazel van GA, Scott M, Eagan RT. The effect of CNS metastases on the survival of patients with small cell cancer of the lung. Cancer 1983; 51: 933-937

5   Hansen HH. Should initial treatment of small cell carcinoma include systemic chemotherapy and brain irradiation? Cancer Chemother Rep 1973; 4: 239-241

6   Ihde DC. Current status of therapy for small cell carcinoma of the lung. Cancer 1984; 54: 2722-2728.

7   Pedersen AG, Kristjansen PEG, Hansen HH. Propylactic cranial irradiation and small cell lung cancer. Cancer Treat Reviews 1988; 15: 85-103.

8   Cox JD, Komaki R, Byhardt RW, Kun LE. Results of whole brain irradiation for metastases from small cell carcinoma of the lung. Cancer Treat Rep 1980; 64: 957-961

9   Baglan RJ, Marks JE. Comparison of symptomatic and prophylactic irradiation of brain metastases from oat cell carcinoma of the lung. Cancer 1981; 47: 41-45

10 Lucas CF, Robinson P, Hoskin PJ, Yarnold JR, Smith IE, Ford HT: Morbidity of cranial relapse in small cell lung cancer and the impact of radiation therapy. Cancer Treat Rep 1986; 70: 565-570

11 Bleyer WA. Hobson's choice in the CNS radioprophylaxis of small cell lung cancer. Int J Radiation Oncology Biol Phys 1988; 15: 783-785.

12 Postmus PE, Haaxma-Reiche H, Vencken LM, Meinesz AF, Sleijfer DTh, Mulder NH. Remission of brain metastases from small cell lung cancer after high-dose chemotherapy. Ann Intern Med 1984; 101: 707

13 Postmus PE, Holthuis JJM, Haaxma-Reiche H, Mulder NH, Vencken LM, Oort van WJ, Sleijfer DTh, Sluiter HJ. Penetration of VP 16-213 into cerebrospinal fluid after high-dose administration. J Clin Oncol 1984; 2: 215-220

14 Viens P, Lagrange JL, Thyss A, Ayela P. Frenay M, Schneider M. Brain metastases of lung cancer: Excessive toxicity of high dose VP 16-213. Eur J Cancer Clin Oncol 1988; 24: 1905-1906

15 Holthuis JJM, Vries EGE de, Postmus PE, Oort van WJ, Verleun H, Hulshoff A, Sleijfer DTh, Mulder NH. Pharmacokinetics of high-dose teniposide (VM26). Cancer Treat Rep 1987; 71: 599-604.

16 Haaxma-Reiche H, Berendsen HH, Postmus PE. Podophyllotoxins for brain metastases of small cell lung cancer. J Neurooncol 1988; 6: 231-232.

17 Order SE, Hellman S, von Essen CF, Kligerman MM. Improvement in quality of survival following whole-brain irradiation for brain metastases. Radiology 1968; 91: 149-153.

18 World health organization. Handbook for reporting results of cancer treatment. WHO offset publ no 48 Nijhoff, The Hague, The Netherlands, 1979.

19 Cohen MH, Creaven PJ, Fossieck BE, Broder LE, Selawry OS, Johnston AV, Williams CL, Minna JD. Intensive chemotherapy of small cell bronchogenic carcinoma. Cancer Treat Rep 1977; 61: 349-354

20 Hansen HH, Muggia FM, Andrews R, Selawry OS. Intensive combined chemotherapy and radiotherapy in patients with non-resectable carcinoma. Cancer 1972; 30: 315-324

21 Hansen HH, Selawry OS, Simon R, Carr OT, van Wyk CE, Tucker RB, Sealy R. Combination chemotherapy of advanced lung cancer: a randomized trial. Cancer 1976; 38: 2201-2207

22 Neystrom ES, Capizzi RL, Rudnick SA, Kirsch M, Delaney D, Kahn L, Lipper S, Carney D. High-dose methotrexate in small cell lung cancer. Lack of efficacy in preventing CNS relapse. Cancer 1983; 51: 1056-1061

23 Cohen MH, Ihde DC, Bunn PA, Fossieck BE, Matthews MJ, Shackney SE, Johnston-Early A, Makuch R, Minna JD. Cyclic alternating combination chemotherapy for small cell bronchogenic carcinoma. Cancer Treat Rep 1979; 63: 163-170

24 Kristjansen PG, Hansen HH. Brain metastases from small cell lung cancer treated with combination chemotherapy. Eur J Cancer Clin Oncol 1988; 24: 545-549

25 Twelves CJ, Souhami RL, Harper PG, Ash CM, Spiro SG, Earl HM, Tobias JS, Quinn H, Geddes DM. Cerebral metastases in small cell lung cancer respond to systemic chemotherapy. Br J Cancer in press

26 Lee JS, Murphy WK, Glisson BS, Dhingra HM, Holoye PY, Hong WK. Primary chemotherapy of brain metastases in small-cell lung cancer. J Clin Oncol 1989; 7: 916-922.

27 Giaccone G, Donadio M, Bonardi GM, Testore F, Calciati A: Teniposide (VM26): an effective treatment for brain metastases of small cell carcinoma of the lung. Eur J Cancer Clin Oncol 1988; 24: 629-631

28 Postmuş PE, Haaxma-Reiche H, Sleijfer DTh, Kirkpatrick A, McVie JG, Kleisbauer JP & the EORTC Lung cancer cooperative group: High dose etoposide for brain metastases of small cell lung cancer. A phase II study. Br J Cancer 1989; 59: 254-256

29 Bork E, Hansen M, Dombernowsky P, Hansen SW, Pedersen AG, Hansen HH. Teniposide (VM-26), an overlooked highly active agent in small cell lung cancer. Results of a phase II trial in untreated patients. J Clin Oncol 1986; 4: 524-527.

30 Pedersen AG, Bork E, Osterlind K, Dombernowsky P, Hansen HH Phase II study of teniposide in small cell carcinoma of the lung. Cancer Treat Rep 1984; 68: 1183-1184.

31 Creaven PJ. The clinical pharmacology of VM 26 and VP 16-213. A brief overview. Cancer Chemother Pharmacol 1982; 7: 133-140.

32 Postmus PE, Berendsen HH, Van Zandwijk N, Splinter TAW, Burghouts JThM, Bakker W. Retreatment with the induction regimen in small cell lung cancer relapsing after an initial response to short term chemotherapy. Eur J Cancer Clin Oncol 1987; 23: 1409-1411.

Chapter 5

ACUTE AND CUMULATIVE EFFECTS OF CARBOPLATIN ON RENAL FUNCTION

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SUMMARY

Carboplatin, a cisplatinum analogue, has no reported nephrotoxicity in phase I/II studies, assessed by creatinine clearance. We prospectively determined renal function in 10 untreated lung cancer patients with normal baseline renal function, treated with carboplatin 400 mg/m² day 1 and vincristine 2 mg day 1 and 8 every 4 wks (max. 5 cycles) by means of clearance studies with ^{125}I-sodium thalamate and ^{131}I-hippurate to determine glomerular filtration rate and effective renal plasma flow respectively. Tubular damage was monitored by excretion of tubular enzymes and relative β_2-microglobulin clearance. During the first course no changes in renal function were seen. After the second course a significant fall in GFR and ERPF started, ultimately leading to a median decrease in GFR of 19.0% (range 6.8-38.7%) and in ERPF of 14% (range 0-38.9%). No increases in the excretion of tubular enzymes or changes in the relative β_2-microglobulin clearances were seen.

We conclude from our data that carboplatin causes considerable loss of renal function. Monitoring renal function in patients treated with multiple courses of carboplatin is warranted.

INTRODUCTION

The introduction of cisplatin (CDDP) in the early seventies resulted in a major step forward in anticancer chemotherapy (1). However, cisplatin has a narrow therapeutic index especially in regard to nephrotoxicity, limiting the clinical utility of this agent (2). Several ways have been employed to overcome this problem. Although therapeutic index of CDDP has improved with the use of such manoeuvres, the drug does remain nephrotoxic (3-8). An alternative approach was the synthesis of analogues of cisplatin with the aim to find Pt-complexes with less nephrotoxicity and more or comparable antitumor activity (9). About 2000 second generation Pt-compounds have been synthesized and screened for cytotoxicity. Only a few have been selected for clinical evaluation, of which carboplatin

(CBDCA, JM8) probably is the most promising. In human and animal studies carboplatin has demonstrated increased hematological toxicity compared to CDDP, but it is less emetogenic and has little or no oto- or neurotoxicity and no nephrotoxicity even in the absence of forced diuresis (10,11). In these studies the renal function was measured by monitoring serum creatinine and creatinine clearances. However, the determination of creatinine as a reflection of the glomerular filtration rate has proven to be a relatively insensitive method to monitor CDDP induced renal damage (12,13). Moreover, using ^{51}Cr EDTA clearances, Calvert et al. (14) were also unable to identify CBDCA as a nephrotoxic drug.

In this study we prospectively determined changes in glomerular filtration rate and effective renal plasma flow by the more sensitive method developed by Donker et al. (15), in 10 patients treated with standard dose carboplatin. The possible tubular damage was monitored by measuring the excretion of tubular enzymes.

PATIENTS AND THERAPY

Ten patients, one female, 9 males, were studied. All had histologically proven lung cancer (8 small cell lung cancer, one squamous cell carcinoma, one endobronchial carcinoid). One patient was pretreated with s.c. infusion of interferon, all others were previously untreated. Their age ranged from 48 to 69 years (mean 58). All had a normal serum creatinine level < 120 μmol/l, were normotensive, not salt restricted, and did not use other potentially nephrotoxic medication.

All patients were treated with carboplatin 400 mg/m^2 day 1 and vincristine 2 mg day 1 and 8 every 4 weeks. Carboplatin was dissolved in 250 ml of glucose 5% and given as a 30 min i.v. infusion on day 1. Vincristine was given as bolus injection. No pre- or posthydration was given.

Seven responding patients received the maximum of 5 courses. The treatment had to be stopped in two patients after three and in one patient after two courses, because of tumor progression.

RENAL FUNCTION STUDIES

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured simultaneously in supine position with radioisotopes. ERPF was determined by measuring the clearance of ^{131}I-hippuran (I x V/P) and GFR by the clearance of ^{125}I-iothalamate (U x V/P) (I = counts per minute of 1 ml sustaining solution, V = infusion volume or urine volume in ml per minute, P = counts per minute in 2 ml of plasma and U = counts per minute in 2 ml urine). After a standard primary dose and sustaining infusion for 2 hours, one-hour clearances were determined for acute effects and 2 hour clearances for cumulative effects. For the latter, values are the mean of two 2 hour clearances, which were corrected for standard body surface area. Errors in GFR introduced by incomplete collection

of urine were corrected with a method previously described. The day to day variation of GFR is $\leq 2\%$ and of ERPF $\leq 5\%$ (15). Filtration fraction (FF) was calculated as the quotient of GFR and ERPF.

Creatinine clearances were also corrected for incomplete collection of urine, using the same method as mentioned above.

These variables were studied before and during four hours after the first carboplatin infusion in order to determine acute effects on renal function. Cumulative effects were also measured during 4 hours 4 weeks after each course, just before the administration of the following course, i.e. on days 29, 57, 85, 113 and 141.

During renal function studies urine was collected hourly for the determination of creatinine, LDH, Alkaline Phosphatase (ALP), gamma-GT and β_2-microglobulin. Serum and urine creatinine, urine, LDH, ALP, gamma-GT, were determined with standard automatic techniques. β_2-Microglobulin concentration in plasma and urine was determined by a radioimmuno sorbent technique according to Evrin et al. (16). Next the ratio of LDH, ALP, gamma-GT respectively and creatinine (U per gram creatinine) were calculated. For β_2-microglobulin the relative clearance in respect to creatinine clearance was calculated.

STATISTICS

Statistical analysis was performed with Wilcoxon's test for paired observations (two-sided); $p < 0.05$ was considered indicative of a significant difference between groups.


RESULTS

1. Acute effects
The pretreatment values (A) of ERPF, GFR and FF of all patients are listed in table I. Also depicted are the nadir values of ERPF and values of GFR and FF corresponding with the nadir values of ERPF after the first carboplatin infusion (B). Also, corresponding creatinine clearances are listed in the table. There were no statistically significant changes in the GFR, either by the radiochemical method or creatinine clearances, and ERPF. For tubulary enzymes no changes were seen. Relative clearance of β_2-microglobulin increased in the 4 hours after carboplatin infusion, but remained within the normal range.

2. Cumulative effects
Absolute and relative changes in GFR and ERPF during the whole treatment are given in table II and figure 1, respectively. Corresponding creatinine clearances are also given. Four weeks after the first administration of carboplatin there is still no significant change in ERPF, GFR and FF, but deterioration of the renal function as measured by the radioisotope clearances occurs after the second course. Out of nine patients still on study 4 weeks after 2 courses, 7 had a fall in GFR > 2% (p < 0.02), median -7.5%, range +1.9%

Table I. **ERPF and GFR (creatinine clearance), all in ml.min^{-1}.1.73 m^2 before (A) and after (B) first carboplatin infusion.**

Patient	ERPF		GFR (creatinine clearance)		FF	
	A	B	A	B	A	B
1	660	601	121 (106)	119 (96)	.18	.20
2	375	395	118 (107)	136 (95)	.30	.35
3	401	397	155 (115)	158 (131)	.39	.39
4	293	309	87 (80)	92 (75)	.28	.30
5	312	322	83 (98)	94 (82)	.27	.29
6	271	235	80 (82)	81 (72)	.30	.34
7	319	315	105 (116)	102 (107)	.33	.32
8	599	517	123 (118)	130 (116)	.21	.23
9	301	309	102 (110)	113 (122)	.34	.37
10	558	526	143 (134)	154 (114)	.26	.29
median	388	358.5	111.5 (108.5)	121.5 (101.5)	0.29	0.31
$\bar{x}$	408.9	398	111.7 (106.6)	117.9 (101)	0.286	0.308
S.D.	143.06	125.88	25.14 (16.5)	26.39 (20.3)	0.0615	0.0596
SE ($\bar{x}$)	45.24	39.81	7.95 (5.21)	8.34 (6.42)	0.0194	0.0188
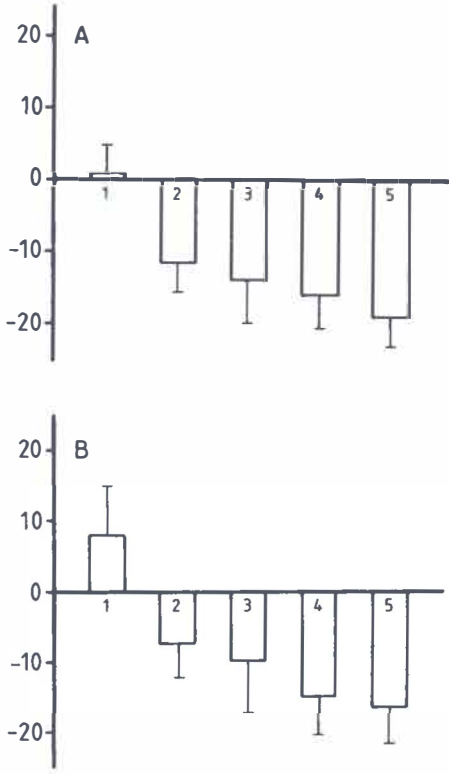
Figure 1. Mean percent changes in GFR (ml.min^{-1}. 1.73 m^2) (A) and ERPF (ml.min^{-1}. 1.73 m^2) (B) after multiple courses of carboplatin (mean ± standard error).

Table II. Absolute changes in GFR (creatinine clearance) and ERPF, all in ml.min^{-1}.1.73 m^2, after multiple courses.

Pat.	Course number					
	0	1	2	3	4	5
GFR (creatinine clearance)						
1	121 (106)	124 (112)	95 (79)	98 (93)	101 (105)	96 (129)
2	118 (107)	137 (124)	113 (101)			
3	155 (115)	116 (100)	99 (116)	97 (109)	94 (179)	95 (68)
4	87 (80)	97 (125)	82 (93)	91 (97)	87 (111)	81 (59)
5	83 (98)	90 (114)	78 (101)			
6	80 (82)	79 (85)	74 (87)	73 (84)	72 (81)	62 (91)
7	105 (116)	102 (113)	107 (72)	101 (109)	89 (107)	85 (91)
8	123 (118)	131 (102)	124 (143)	120 (145)	119 (123)	114 (127)
9	102 (110)	116 (120)				
10	143 (134)	111 (90)	110 (90)	98 (79)	103 (50)	122 (116)
Median	111.5 (110)	116 (112.5)	99 (93)	98 (97)	94 (111)	95 (91)
$\bar{x}$	111.7 (106.5)	110.3 (108.5)	98 (99)	96.9 (102.3)	95.0 (108)	93.6 (97)
S.D.	25.14 (16.5)	18.35 (13.8)	17.20 (22.5)	13.90 (22.0)	14.75 (39.5)	10.26 (27.8)
S.E. ($\bar{x}$)	7.95 (5.21)	5.80 (4.37)	5.73 (7.49)	5.25 (8.31)	5.58 (14.94)	7.66 (10.5)
ERPF						
1	660	540	396	413	423	403
2	375	492	424			
3	401	470	407	360	320	310
4	293	31	283	350	288	290
5	312	76	302			
6	271	260	60	254	249	234
7	319	354	396	372	330	314
8	599	554	99	531	558	555
9	301	452				
10	558	385	374	348	382	398
Median	388	418.5	396	360	330	314
$\bar{x}$	408.9	421.4	371.2	375.4	364.3	357.7
S.D.	143.06	95.50	76.30	83.67	102.90	105.41
S.E. ($\bar{x}$)	45.24	30.20	25.43	31.63	38.89	39.84

to -36.1%. In three patients ERPF decreased > 5% as opposed to pretreatment values; median -3.4%, range +24.1% to -40.0% (p < 0.05). The ultimate decrease after 5 courses (n = 7) ranges from 6.8% to 38.7% for GFR (median 19.0%) (p < 0.02) and for ERPF 1.6% to 38.9% (median 14%) (p < 0.02). These changes could not be explained by alteration in body weight of individual patients.

Although creatinine clearances showed a tendency to decrease after course two, this change was not significant (p > 0.1). Moreover, 4 weeks after the fourth course creatinine clearances returned to baseline values, with the exception of those in patient number 10. After 5 courses no significant difference as opposed to pretreatment values were found.

During the observation period no significant changes in serum creatinine were found.

In regard to tubular enzymes and relative ß$_2$ clearance we could not detect significant changes. Also, none of the patients developed proteinuria.

DISCUSSION

The most serious side effect of cisplatin is nephrotoxicity. After multiple courses of CDDP, a decrease of about 40% in creatinine clearance has been reported (12,17). The study of Meijer et al. (12) using the same method as used in this report, showed a median decrease in GFR and ERPF of both 23% after induction chemotherapy containing CDDP for non-seminomatous testicular cancer. The cisplatin analogue carboplatin has no reported nephrotoxicity at conventional dose levels. The reduced protein binding (18,19) and greater stability of carboplatin in body fluids and therefore increased renal excretion compared to cisplatin are supposed to account for the absence of nephrotoxicity (20,21). Also, in animal models, carboplatin enhanced nuclear protein phosphorylation in tumor cells more than CDDP did, but caused much less protein phosphorylation in the normal liver and kidney cells. This suggests some selective toxicity towards tumor cells and may in part explain the decreased nephrotoxicity of carboplatin (22). Therefore, carboplatin has been recommended as an alternative to cisplatin in patients with impaired renal function or in those who can not receive the hydration required for conventional cisplatin administration (23).

However, there have been sporadic observations of renal function deterioration after multiple courses of carboplatin (11,14,24,25). Also, the high dose (≥ 800 mg/m^2) study of Gore et al. (26) showed a fall in GFR of > 25% in 55% of courses, as measured by ^{51}Cr-EDTA clearances.

Since vincristine has no reported nephrotoxicity (27,28), we conclude from the data of our study that carboplatin has a cumulative dose related nephrotoxic effect, as there was no decrease in renal function parameters after the first course, but an impressive fall in GFR and ERPF after the second course, ultimately leading to a decrease of 38% after 5 courses in some patients. The fall in GFR may be clinically important, because the degree of myelosuppression probably depends on GFR (29-31). In our patients, however, we did not find cumulative hematological toxicity despite this decrease in GFR. Even the patient with a 38% reduction in GFR did not have severe myelosuppression.

This study also shows the superiority of the radiochemical method to determine GFR as opposed to creatinine clearance in order to monitor Pt induced renal damage. The latter method failed to detect a significant change in GFR after multiple courses of carboplatin. A possible explanation for this finding is provided by Meyer et al (12). A significant fall in GFR (median 23%) was seen after combination chemotherapy containing CDDP, without a rise in serum creatinine. They suggested that the chemotherapy interfered with enzymatic systems required for creatinine production.

The mode of action of Pt-induced nephrotoxicity remains unknown. Offerman et al. (32) have shown that the acute effect of CDDP on renal function is a fall in ERPF preceding a similar change in GFR. Also, in experimental models of renal failure following intoxication with heavy metals, in the initial phase a reduction of renal blood flow can be found. In our study no such phenomenon could be found, as during the first course neither a fall in GFR nor in ERPF was seen. Since a simultaneous decrease in both GFR and ERPF occurred 4 weeks after course two, we can only speculate, but not exclude, whether such a sequence of events did take place. Also, the intracellular presence of reactive Pt-compounds in the kidney is suggested to relate to this toxicity (20,33). Therefore tubular damage might play an important role in Pt-induced nephrotoxicity. The reported renal uptake of carboplatin does not differ substantially from that for CDDP (10,34). CDDP induces tubular damage in most patients but we could not find signs of tubular damage after carboplatin administration as measured by the urinary excretion of tubular enzymes, because of infrequent sampling. For the evaluation of tubular damage timing of specimen collection plays an important role (35). As reported for cisplatin (36) and carboplatin (18), urinary enzymes can peak as late as several days after the administration of the drug. Shillen et al. (37) collected weekly specimen for the evaluation of excretion of urinary protein and enzymes in patients receiving multiple courses of carboplatin 400 mg/m². In some of their patients urinary enzymes peaked two weeks after the administration of the drug. Therefore, our results might be misleading in that they were obtained for a period of only four hours after administration.

We conclude from our data that carboplatin exerts dose related cumulative renal damage. Careful monitoring, especially with regard to myelosuppression, in patients with impaired renal function or those pretreated with Pt containing regimen is therefore warranted. The observed reduction in renal function after carboplatin is in the same range compared to patients treated with cisplatin with saline diuresis (12). Although it has not been excluded that hyperhydration during carboplatin treatment could prevent the nephrotoxic effects, the value of administering this cytostatic drug on an out-patient base would disappear.

REFERENCES

1. Carter, S.K. (1984). Cisplatin - past, present and future. In: Platinum coordination complexes in cancer chemotherapy, Hacker, M.P., Douple, E.B. & Krakoff, I.H. (eds) p. 359. Martinus Nijhoff Publishing: Boston/The Hague, Dordrecht, Lancaster.

2. Madias, N.E., Harrington, J.T. (1978). Platinum nephrotoxicity. Am.J.Med., 65, 307.
3. Al-Sarraf, M., Fletcher, W., Oishi, H., (1983). Cisplatin hydration with and without mannitol diuresis in refractory disseminated malignant melanoma: a southwest oncology group study. Cancer Treat.Rep., 66, 31.
4. Ozols, R.F., Cordon, B.J., Jacob, J., Wesley, M.M., Ostchega, Y., Young, R.C. (1984). High-dose cisplatin in hypertonic saline. Ann.Int.Med., 100, 19.
5. Markman, M., Cleary, S., Howell, S.B. (1985). Nephrotoxicity of high-dose intracavitary cisplatin with intravenous thiosulfate protection. Eur.J. Cancer Clin.Oncol., 21, 1015.
6. Bodenner, D.L., Dedon, P.C., Keng, P.C., Katz, J.C., Borch, R.F. (1986). Selective protection against cis-diamminechlorideplatinum (II)-induced toxicity in kidney, gut and bone marrow by diethyldithiocarbamate. Cancer Res., 46, 2751.
7. Elferink, F., van der Vijgh, W.J.F., Klein, I., Pinedo, H.M. (1986). Interaction of cisplatin and carboplatin with sodium thiosulfate: reaction rates and protein binding. Clin.Chem., 32, 641.
8. Offerman, J.J.G., Mulder, N.H., Sleijfer, D.Th. (1985). Influence of captopril on cis-diamminedichloro-platinum induced renal toxicity. Am.J.Nephrol., 5, 433.
9. Burchenal, J.H., Kalaher, K., Dew, K., Lokys, L. (1979). Rationale for development of platinum analogs. Cancer Treat.Rep., 65, 1493.
10. Lelieveld, P., van der Vijgh, W.J.F., Veldhuizen, R.W. (1984). Preclinical studies on toxicity, antitumour activity and pharmacokinetics of cisplatin and three recently developed derivates. Eur.J.Cancer Clin. Oncol., 20, 1087.
11. Van Glabbeke, M., Renard, J., Pinedo, H.M. (1988). Iproplatin and carboplatin induced toxicities: overview of phase II clinical trial conducted by the EORTC Early Clinical Trials Cooperative Group (ECTG). Eur.J.Cancer Clin.Oncol., 24, 255.
12. Meijer, S., Sleijfer, D.Th., Mulder, N.H. (1983). Some effects of combination chemotherapy with cis-platinum on renal function in patients with nonseminomatous testicular carcinoma. Cancer, 51, 2035.
13. Daugaard, G., Rossing, N., Rorth, M. (1988). Effects of cisplatin on different measures of glomerular function in the human kidney with special emphasis on high dose. Cancer Chemother.Pharmacol., 21, 163.
14. Calvert, A.H., Harland, S.J., Newell, D.R., (1982). Early clinical studies with cis-diammine-1, 1-cyclobutane dicarboxylate platinum II. Cancer Chemother.Pharmacol., 9, 140.
15. Donker, A.J.M, , van der Hem, G.K., Sluiter, W.J., Beekhuis A. (1977). A radioisotope method for simultaneous determination of the glomerular filtration rate and the effective renal plasma flow. Neth.J.Med., 20, 97.
16. Evrin, P.E., Peterson, P.A., Wide, L., Berggard, I. (1971). Radioimmunoassay of β_2 microglobulin in human biological fluids. Scand.J.Clin.Lab.Invest., 28, 439.
17. Dentino, M., Luft, F.C., Yum, M.N., Williams, S.D., Einhorn, L.H. (1978). Long term effect of cis-diamminochloride platinum (CDDP) on renal function and structure in man. Cancer, 41, 1274.
18. Van Echo, D.A., Egorin, M.J., Whitacre, M.Y., Olman, E.A., Aisner, J. (1984). Phase I clinical and pharmacologic trial of carboplatin daily for 5 days. Cancer Treat.Rep., 68, 1103.
19. Gaver, R.C., George, A.M., Dab, G. (1987). In vitro stability, plasma protein binding and blood cell partitioning of 14 C carboplatin. Cancer Chemother. Pharmacol., 20, 271.
20. Harland, S.J., Newell, D.R., Siddik, Z.H., Chadwick, R., Calvert, A.H., Harrap, K.R. (1984). Pharmacokinetics of cis-diammine-1, 1-cyclobutane dicarboxylate platinum (II) in patients with normal and impaired renal function. Cancer Res., 44, 1693.
21. Sharma, H., Thatcher, N. Bear, J. (1983). Blood clearance of radioactively labelled cis-diammine 1, 1-cyclobutane dicarboxylate platinum (II) (CBDCA) in cancer patients. Cancer Chemother. Pharmacol., 11, 5.

22. Harrap, K.R., Jones, M., Wilkinson, C.R. (1980). Antitumor, toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Cisplatin current status and new developments. Prestayko, A.W., Crooke, S.T. & Carter, S.K. (eds). Academic Press: London.
23. Von Hoff, D.D. (1987). Whither carboplatin - a replacement for or an alternative to cisplatin. J.Clin.Oncol., 5, 169 (editorial).
24. Rozenczweig, M., Nicaise, C., Beir, M. (1983). Phase I study of carboplatin given on a five-day intravenous schedule. J.Clin.Oncol., 1, 621.
25. Leyvraz, S., Ohnuma, T., Lassus, M., Holland, J.F. (1985). Phase 1 study of carboplatin in patients with advanced cancer, intermittent intravenous bolus and 24-hour infusion. J.Clin.Oncol., 3, 1385.
26. Gore, M.E., Calvert, A.H., Smith, I.E. (1987). High dose carboplatin in the treatment of lung cancer and mesothelioma: a phase 1 dose escalation study. Eur.J.Cancer Clin.Oncol., 23, 1391.
27. Schilsky, R.L. (1982). Renal and metabolic toxicities of cancer chemotherapy. Sem.Oncol., 9, 75.
28. Weis, R.B., Poster, D.S. (1982). The renal toxicity of cancer chemotherapeutic agents. Cancer Treat.Rev., 9, 37.
29. Egorin, M.J., Van Echo, D.A., Olman, E.A., Whitacre, M.Y., Forrest, A., Aisner, J. (1985). Prospective validation of a pharmacologically based dosing scheme for the cis-diammine dichloroplatinum (II) analogue cisdiammine (1, 1) cyclobutane-dicarboxylatoplatinum. Cancer Res., 45, 6502.
30. Fish, R.G., Shelley, M.D., Griffith, H., Adams, M. (1987). (Letter). Cancer Res., 47, 3606.
31. Egorin, M.J., Van Echo, D.A., Tipping, S.J. & 4 others (1984). Pharmacokinetics and dosage reduction of cis-diammine(1, 1-cyclobutanedi-carboxylato) platinum in patients with impaired renal function. Cancer Res., 44, 5432.
32. Offerman, J.J.G., Meyer, S., Sleijfer D.Th. (1984). Acute effects of cisdiammine-dichloroplatinum (CDDP) on renal function. Cancer Chemother.Pharmacol., 12, 36.
33. Stewart, D.J., Mikhael, N.Z., Nanji, A.A. (1985). Renal and hepatic concentrations of platinum: relationship to cisplatin time, dose and nephrotoxicity. J.Clin.Oncol., 3, 1251.
34. Owens, S.E., Thatcher, N., Sharma, H. (1985). In vitro distribution studies of radioactively labelled platinum complexes: cis-dichloro-diammine platinum (II), cis-trans-dichloro-dihydroxy-bis-(isopropylamine) platinum (IV), cis-dichloro-bis-cyclopropylamine platinum (II) and cis-diammine 1, 1-cyclobutanedicarboxylate platinum (II) in patients with malignant disease, using a gamma camera. Cancer Chemother.Pharmacol., 14, 253.
35. Goren, M.P., Forastiere, A.A., Wright, R.K. (1987). Carboplatin (CBDCA), iproplatin (CHIP) and high dose cisplatin in hypertonic saline evaluated for tubular nephrotoxicity. Cancer Chemother.Pharmacol., 19, 57.
36. Goren, M.P., Wright, R.K., Horowitz, M.E. (1986). Cumulative renal tubular damage associated with cisplatin nephrotoxicity. Cancer Chemother. Pharmacol., 18, 69.
37. Shillen, A.W., Buamah, P.K., Cantwell, B.M.J., Cornell, C., Hodson, A.W., Harris, A.L. (1988). Urinary protein and enzyme excretion in patients receiving chemotherapy with the cis-platin analogs carboplatin (CBDCA, JM8) and iproplatin (CHIP, JM9). Cancer Chemother. Pharmacol., 22, 228.

Chapter 5a

CARBOPLATIN AND RENAL DYSFUNCTION

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To the Editor:

Reed and Jacob report on renal toxicity following high dose carboplatin (800mg/m^2) in cisplatin pretreated patients with refractory ovarian cancer(1). In three patients a substantial decrease in creatinine clearance (CrCl) was seen. In nine additional patients, who received vigorous intravenous hydration no reductions in CrCl occurred. Mulder et al.(2) communicated a case history of a patient who developed renal failure after a cumulative dose of carboplatin of 750 mg/m^2 in three days. This patient was also pretreated with a cisplatin containing regimen. However, carboplatin has proven to be also nephrotoxic in untreated patients. In the high dose study of Gore et al.(3) a fall in glomerular filtration rate (GFR) >25% was noticed in 10/20 courses of carboplatin 800-1600 mg/m^2, measured by [^{51}Cr]EDTA clearances. We prospectively determined GFR and effective renal plasma flow (ERPF) in ten patients with lung cancer, treated with carboplatin 400 mg/m^2 and vincristine 2 mg day 1 and 8 every 4 weeks. Renal function was assessed after each course with a radiochemical method.(4). No hydration regimen were administered. A significant reduction in both GFR and ERPF was seen after 2 courses, ultimately leading after 5 courses to a median decrease in GFR of 19% and in ERPF of 14%. Interestingly, the cumulative carboplatin dose associated with the onset of the fall in renal function parameters was 800 mg/m^2, i.e. the same dose as used in the report of Reed and Jacob(1). The change in GFR and ERPF did not lead to an analogous fall in CrCl. This may account for the fact that in various phase I and II studies carboplatin was not identified as a nephrotoxic agent.

The intracellular presence of reactive Pt-compounds is thought to be responsible for Pt induced nephrotoxicity. Pt-DNA adduct formation in the kidney by carboplatin superimposed upon already existing identical lesions due to previous cisplatin treatment may well account for the nephrotoxicity observed by Reed and Jacob. There seems to be a treshold for this mechanism, as the cumulative carboplatin dose has to exceed at least 750 mg/m^2 before nephrotoxicity becomes apparent. Increasing impairment of renal function by this mode of action could be augmented by the often unavoidable use of other known nephrotoxic agents or in patients with pre-existing decreased renal function not due to cisplatin treatment. Therefore, we feel that carboplatin should be administered with caution to all patients with compromised renal function. Whether hyperhydration during carboplatin treatment could ameliorate nephrotoxic effects is not yet established. However, such an approach certainly would restrict the use of this activ anti-cancer agent on outpatient base.

REFERENCES

1. Reed E, Jacob J. Carboplatin and renal dysfunction. Ann. Intern. Med. 1989;110;409. (Letter)
2. Mulder POM, Sleijfer DTh, de Vries EGE, Uges DRA, Mulder NH. Renal dysfunction following high-dose carboplatin treatment. J. Cancer Res. Clin. Oncol. 1988;114;212-214.
3. Gore ME, Calvert AH, Smith IE. High dose carboplatin in the treatment of lung cancer and mesothelioma: a phase I dose escalation study. Eur. J. Cancer Clin. Oncol. 1987;23;1391-1397.
4. Sleijfer DTh, Smit EF, Meijer S, Mulder NH, Postmus PE. Acute and cumulative effects of carboplatin on renal function. Br. J. Cancer in press.

Chapter 6

A PHASE TWO STUDY OF CARBOPLATIN AND VINCRISTINE IN PREVIOUSLY TREATED PATIENTS WITH SMALL CELL LUNG CANCER

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SUMMARY

Twenty-eight previously treated patients with small cell lung cancer were treated at relapse with carboplatin 400 mg/m² and vincristine 2 mg day 1 and 8 every 4 weeks. Ten partial responses (PR)(37%) were seen, no complete responses. Median survival after start of second line treatment was 120 (range 39-503) days. Toxicity of this regimen was moderate, including WHO grade 3/4 myelosuppression in 26% of courses (n=66). Eight PR's were seen in a subgroup of 22 patients relapsing less than three months off first line treatment. The responses seen in this group of patients may be due to absence of cross resistance between the regimen used.

INTRODUCTION

Small cell lung cancer (SCLC) is a rapidly progressive and ultimately fatal disease. Combination chemotherapy is the treatment of choice resulting in response rates up to 95%; however, only 5-15% of the patients with limited disease (LD) survive beyond 5 years (8) and patients with extensive disease (ED) are rarely cured (10,12). This depressing fact is caused by the development of resistance to the initially effective drugs in relapsing patients. Therefore, there is a great need for active second line combinations of cytotoxic agents.

Carboplatin (CBDCA) is a second generation platinum analogue with little evidence of nephrotoxicity at dose levels used in phase II studies (19). Compared to its parent compound cisplatin, it is less emetogenic, neuro- and ototoxic, while myelosuppression, in particular thrombocytopenia, was found to be dose limiting (2,3,6). In contrast to cisplatin (4), carboplatin has shown high activity against SCLC, with response rates, either as single agent or in combination chemotherapy, ranging from 62%-95% in previously untreated patients (1,9,17,18).

In this study we report on the efficacy and toxicity of carboplatin in combination with vincristine, in previously chemotherapeutic treated patients with SCLC. The latter drug was selected for its non myelosuppressive character.

PATIENTS AND METHODS

Between June 1986 and October 1988 28 consecutive patients were entered in the study. Eligibility criteria were histological proof of SCLC, at start of treatment WBC > $3.0 \times 10^9/l$, platelets > $100 \times 10^9/l$, haemoglobin > 6.8 mmol/l (unless lower values were caused by involvement of bone marrow), serum creatinine < 120 mmol/l, bilirubin < 25 μmol/l, no signs of central nervous system metastases. Informed consent was obtained from all patients, according to local medical ethical committee regulation. For patients characteristics see table I.

Table I.   Patient characteristics.

	number of patients
Male : Female	26 : 2
Median age (range) years:	62 (46-77).
LD : ED	8 : 20
ECOG PS at start of therapy:	
0	2
1	7
2	8
3	10
4	1
Time off first line treatment (months).	
<1	15
1-3	7
>3	6

Their median age was 62 years (range 46-77 years). At start of chemotherapy 8 patients had LD, i.e. confined to one hemithorax and supraclavicular nodes, 20 had ED, i.e. beyond these borders. All patients had relapsed after chemotherapy. First line treatment consisted for ten patients of orally administered etoposide (E) alone, and for eighteen patients of cyclophosphamide (C), doxorubicin (D) and E intravenous combination chemotherapy. Responses to first line treatment are listed in table II. Fifteen patients had shown a tumor relapse during induction treatment, i.e. within one month after the last course of induction chemotherapy. Seven patients had a treatment free interval between one and three months, whereas for six patients this was more than three months (see table I).

Carboplatin 400 mg/m² was dissolved in 250 ml glucose 5% and administered as a 30 min intravenous infusion on day 1. Vincristine 2 mg was given by bolus injection on day 1 and 8. Courses were repeated every 4 weeks until disease progression for a maximum of 5 cycles. Dose adjustments were made for myelosuppression (carboplatin) or neurotoxicity (vincristine).

Toxicity and response were measured according to WHO criteria (21) on day 21 after the start of every course. A complete response (CR) was defined as disappearance of all

measurable and evaluable lesions; a partial response (PR) was a >50% reduction in the product of the greatest tumor diameter and its perpendicular for all measurable lesions. The term stable disease (SD) was applied if there was a <50% decrease in measurable disease, or a <25% increase of tumor size. Progressive disease (PD) was defined as an increase by >25% in the product of perpendicular diameters of measurable lesions or the occurence of any new lesions. Survival was measured from the start of second line treatment. Patients were considered evaluable for survival if they completed at least one cycle of chemotherapy.

RESULTS

Response and survival (table II)
One patient had no measurable or evaluable tumor localisations, leaving 27 patients evaluable for response. There were no CR's. Ten patients had a PR (37%), including 8 patients (36%) in the group relapsing within three months off induction treatment. Ten patients had SD (37%), including two patients who had marked diminishment of pleural fluids with subjective improvement without fulfilling criteria for response. Seven patients had PD, three of them died during the first cycle of chemotherapy due to tumor progression. Median survival time (MST) for the whole group (25 patients evaluable) was 120 (range 39-503) days. MST for the 22 patients relapsing within three months off induction chemotherapy was 126 (range 53-503) days.

Table II. **Response to first line treatment (FLT) and response to Carboplatin Vincristine (CV) combination.**

FLT (no patients)	Response to FLT			Response to CV			
	CR	PR	SD	PR	SD	PD	NE
CDE (5)	1	4	–	2	1	2	–
CDE*(13)	1	11	1	4	5	4	–
E (1)	–	1	–	–	–	–	1
E*(9)	1	6	2	4	4	1	–

Abbreviations used: C=Cyclophosphamide, D=Doxorubicin, E=Etoposide. ,
*relapsing during treatment or within three months off first line chemotherapy (see text).

Toxicity
The total number of courses was 66, median 3. Thrombocytopenia and leucocytopenia WHO grade 3/4 occurred in 26% of courses. Dose reduction of carboplatin was necessary in three patients. Peripheral neuropathy was seen in 13 patients, leading to dose reduction of vincristine in 8 patients. Symptomatic therapy for nausea and vomiting was given to all

patients, usually with fair success as grade 2 gastrointestinal toxicity was seen in only 16% of courses. None of the patients had a significant rise in serum creatinine, none complained of hearing loss.

DISCUSSION

Postmus et al (14) found a total response rate of 62 % with 13% CR in a group of previously untreated patients treated with the same regimen as in the reported study. In previous studies of carboplatin as single agent in pretreated patients response rates varied between 0 and 19% (16,22). Our results show that the combination of carboplatin and vincristine is an active regimen in pretreated patients with SCLC.

Of particular interest is the 37% response rate in this group of pretreated patients. As Vincent et al (20) pointed out, progression after first line chemotherapy does not necessarily indicate resistance in a clinical context, as patients in their material responded to rechallenge with the same regimen used as initial chemotherapy. The duration of a primary response might be of paramount importance for results of second line treatment; all patients reported by them had durations of response of at least three months (range 3-30 months). Giaccone et al (7) retreated patients with the same regimen used as induction chemotherapy after a treatment free period of at least 14 weeks. They reported 2 CR and 4 PR in a group of 13 selected patients. Finally Postmus et al (13) found that patients who had a first response of more than 34 weeks had a significantly higher probability of achieving a second response as patients who had a first response of less than 34 weeks. Also, response duration after second line treatment was clearly influenced by time off treatment. From these data it seems reasonable therefore to consider patients who relapse within three months off treatment as clinically resistant to the initially given chemotherapy. Consequently, in this group of patients it is not necessary to demonstrate resistance to the initially given chemotherapy before evaluating potentially non cross resistant regimen (20). Because 22 of the patients reported in this study relapsed during or within three months off induction chemotherapy, the responses seen in this group may be due to absence of cross resistance between the drugs given initially and at relapse. One might argue that responses seen after single agent chemotherapy as first line treatment, i.e. etoposide alone, are not surprising. However, MST after second line treatment with carboplatin and vincristine was only slightly worse for patients initially treated with combination chemotherapy, i.e. CDE, compared to the first group. MST for patients who received etoposide alone as first line treatment was 136 (range 60-503) days, for the latter group this was 116 (range 53-362) days.

A disappointing feature of the responses seen in this group of patients is that they were neither complete nor of long duration. This is common to almost all studies of second line chemotherapeutic treatment of SCLC patients. Probably the prognosis of patients with SCLC is denominated by the most resistant cell clones in a given tumor. Completely non cross resistant regimen are needed to eradicate also these cell clones; comparable to the

alternating regimen MOPP/ABVD in Hodgkin's disease (15). For SCLC alternating che-
motherapy has been disappointing so far. However, most studies that have been reported
used regimen that had not been sufficiently evaluated for their supposed non cross resi-
stance (11). From our results it is obvious that carboplatin is a good candidate for a regi-
men to be alternated with CDE. This is supported by the fact that CDE produces responses
in patients who relapse on carboplatin based regimen; the EORTC lung cancer study
group reported 14 major responses in 29 patients previously treated with the latter regi-
men(5).

As anticipated, toxicity of the regimen was predominantly myelosuppression, espe-
cially thrombocytopenia. However, there were no toxic deaths and only one patient had
to be hospitalised because of bleeding episodes. None of the patients experienced aplasia
related sepsis. Dose reductions were given in a minority of the patients. Nausea and vomi-
ting were seen in a majority of the patients, usually responsive to simple anti emetic regi-
men. No evidence of nephrotoxicity was seen as measured by serum creatinine.
Neurotoxicity, probably due to vincristine was encountered in 13 patients.

In summary, this study shows that the combination of carboplatin and vincristine is an
active regimen in pretreated patients with SCLC, which may be -partially- non cross resi-
stant to CDE or E alone. Toxicity of this regimen is moderate.

REFERENCES

1. Bishop J.F., Raghavan D., Stuart-Harris R., Morstyn G., Aroney R., Kefford R., Yuen K., Lee
    J., Giamoutsous P., Olver J.N., Zalcberg J., Ball D., Bull C., Fox R. Carboplatin (CBDCA, JM8)
    and Vp 16-213 in previously untreated patients with small cell lung cancer. J. Clin. Oncol. 1987,
    5, 1574-1578.
2. Calvert A.H., Harland S.J., Newell D.R., Siddik Z.H., Jones A.C., McElwain T.J., Raju S.,
    Wiltshaw E., Smith I.E., Baker J.M., Peckham M.J., Harrap K.R., Early clinical studies with cis-
    Diammine-1, 1-Cyclobutane Dicarboxylate Platinum II. Cancer Chemother. Pharmacol. 1982,
    9, 140-147.
3. Curt G.A., Grygiel J.J., Corden B.J. A Phase I and pharmacokinetic study of diammine cyclo-
    butane-dicarboxylate platinum. (NSC 241240) Cancer Res. 1983, 43, 4470-4473.
4. Dombernowsky P., Sorensen S., Aisner J., Hansen H.H. cis-Dichlorodiammineplatinum (II) in
    small cell anaplastic bronchogenic carcinoma: A phase II study. Cancer Treat. Rep. 1979, 63,
    543-545.
5. EORTC lung cancer cooperative group. Evaluation of non-cross resistance of carboplatin con-
    raining regimen with cyclophosphamide, doxorubicin and etoposide in patients with small cell
    lung cancer. EORTC-study 08862. abstr. 6.1.63, Interlaken, 1988.
6. Foster B.J., Clagget - Carr K., Leyland Jones B., Hoth D. Results of NCI-sponsored phase I
    trials with Carboplatin. Cancer Treat. Rev. 1985, 12 (suppl A), 43-49.
7. Giaccone G., Ferrati P., Donadio M., Testore F., Calciati A. Reinduction chemotherapy in small
    cell lung cancer. Eur. J. Cancer Clin. Oncol. 1987, 23, 1697-1699.
8. Ianuzzi M.C., Scoggin C.H. Small cell lung cancer, state of the art. Am. Rev. Resp. Dis., 1986,
    134, 593-608.

9. Jacobs R.H., Bitran J.D., Deutsch M., Hoffman P.C., Sinkule J., Purl S., Golomb H.M. Phase II study of Carboplatin in previously untreated patients with metastatic small cell lung carcinoma. Cancer Treat. Rep. 1987, 71, 311-312.
10. Johnson B.E., Ihde D.C., Bunn P.A., Patients with small cell lung cancer treated with combination chemotherapy with or without irradiation. Ann. Int. Med. 1985, 103, 430-438.
11. Johnson D.H., Greco F.A. Treatment of small cell lung cancer: another study on alternating chemotherapy.Eur J. Cancer Clin. Oncol., 1987, 23, 1577-1579.
12. Livingston R.B., Stephens R.L., Bonnet J.D., Long term survival and toxicity in small cell lung cancer. Am. J. Med. 1984, 77, 415-417.
13. Postmus P.E., Berendsen H.H., van Zandwijk N, Slinter T.A.W., Burghouts J.Th.M., Bakker W., the EORTC Lung Cancer Cooperative Group. Retreatment with the induction regimen in small cell lung cancer relapsing after an initial response to short term chemotherapy. Eur. J. Cancer Clin. Oncol. 1987, 23, 1409-1411.
14. Postmus P.E., Kirkpatrick A., Dalesio O., Palmen F.J., Carney D.N., Festen J., Vendrik C., Roozendaal K., Burghouts J., Planting E., Quoix E., Slinter T.A.W., McVie J.G. Two carboplatin containing regimen for small cell lung cancer: preliminary results of a randomized phase II trial. Cancer Treat. Rev., Vol 15 (suppl B) 1988, 41-44.
15. Santoro A., Bonadonna G., Bonfante V., Valagussa P. Alternating drug combinations in the treatment of advanced Hodgkin's disease. New Eng. J. Med., 1982, 306, 770-775.
16. Smith I.E., Harland S.J., Robinson B.A., Evans B.D., Goodhart L.C., Calvert A.H., Yarnold J., Glees J.P., Baker J., Ford H.T. Carboplatin: a very active new cisplatin analog in the treatment of small cell lung cancer. Cancer Treat. Rep. 1985, 69, 43-46.
17. Smith I.E., Evans B.D., Gore M.E., Vincent M.D., Repetto L., Yarnold J.R., Ford H.T. Carboplatin (Paraplatin, JM8) and Etoposide (VP-16) as first line combination therapy for small cell lung cancer. J. Clin. Oncol. 1987, 5, 185-189.
18. Smith I.E., Perrent V. Carboplatin, Vp-16 and Ifosfamide intensive chemotherapy for small cell lung carcinoma. Proc. ASCO, 1987, 6, 179.
19. Van Glabbeke M, Renard J, Pinedo H.M., Cavalli F, Vermorken J, Cessa C, Abele R, Clavel M, Monfardini S. Iproplatin and Carboplatin induced toxicities: overview of phase II clinical trials conducted by the EORTC Early Clinincal Trials Cooperative Group. Eur. J. Cancer Clin. Oncol. 1988, 24, 255-262.
20. Vincent M., Evans B., Smith I.E. First line chemotherapy rechallenge after relapse in small cell lung cancer. Cancer Chemother. Pharmacol. 1988, 21, 45-48.
21. WHO Handbook for reporting results of cancer treatment. WHO Offset Publication no.48, Nijhoff Den Haag, 1978.
22. Winkler C.F., Batist G, Veach S.R., Eddy J.L., Mulshine J.L., Ihde D.C. Phase II study of cis-diammine-1, 1 cyclobutane dicarboxylate platinum II (CBDCA, NSC 241240) in non small cell and small cell lung cancer. Proc. ASCO 1985, 4, 186.

Chapter 7

A PHASE II STUDY OF CARBOPLATIN AND VINCRISTINE IN NON-SMALL CELL LUNG CANCER

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SUMMARY

Thirty patients with metastatic non-small cell lung cancer (NSCLC) were treated with carboplatin 400 mg/m^2 and vincristine 2 mg days 1 and 8 every 4 weeks. Five partial responses (16.7%) were seen, no complete responses. Median survival was 6 months. Toxicity was mainly non-haematological, with nausea and vomiting WHO grade II/III in 63% of courses. The incidence of myelosuppression was low: only 4% of courses were associated with WHO grade III/IV myelosuppression. Peripheral neuropathy was seen in 50% of patients. It is concluded that the role of carboplatin and vincristine in non-small cell lung cancer remains to be defined.

INTRODUCTION

The results of polychemotherapy in non small cell lung cancer (NSCLC) are still unsatisfactory. During the last two decades various combination chemotherapy regimen have been studied in phase II setting. Initial reports showed for some of these combinations considerable response rates. However, these results could often not be confirmed in later studies. In the literature the overall response rate seems to be between 20 and 40% with few complete responnders (1). Survival is only marginally improved by chemotherapy when compared to supportive care only (2-4). It seems therefore worthwhile to test new drugs or analogues of currently available drugs with some activity against NSCLC. Carboplatin (cis-Diammino-1,1-cyclobutanedicarboxylato-platinum-II) is the one platinum analogue that has received widespread clinical testing in malignancies that are normally treated with cisplatin but also in others (5). Initially, carboplatin was selected out of over 2000 newly synthesized second generation platinum analogues because, in comparison to cisplatin, it demonstrated lesser toxicity and comparable antitumor activity in preclinical testing systems (6). In the clinic, carboplatin is less emetogenic, nephro-, neuro- and ototoxic, while myelosuppression, in particular thrombocytopenia, was found to be dose limiting (7). The reduced toxicity is in part explained by the pharmacological profile

of the drug (8), but also some selective toxicity towards tumor cells compared to liver and renal cells may play a role (9). Single agent cisplatin ranks among the six (out of fifty tested) drugs with significant anti tumor activity in more than 15% of patients with NSCLC entered in phase II trials (10).

In this study we investigated the efficiacy and toxicity of carboplatin in combination with vincristine, in patients with unresectable or metastatic NSCLC. The latter drug was selected for its non myelosuppressive character.

PATIENTS AND METHODS

Between June 1986 and October 1988 30 consecutive patients were entered in the study. Eligibility criteria were histological proof of NSCLC, at start of treatment WBC > $3.0 \times 109/l$, platelets > $100 \times 109/l$, haemoglobin > 6.8 mmol/l (unless lower values were caused by involvement of bone marrow), serum creatinine < 120 mmol/l, bilirubin < 25 μmol/l, no signs of central nervous system metastases. Informed consent was obtained from all patients, according to local medical ethical committee regulation. For patients characteristics see table I.

Their median age was 54 years (range 40 - 69 years). At start of chemotherapy 18 patients were classified as stage III disease, 13 had stage IV disease. Nine patients had received radiotherapy to primary tumor localizations, two patients had received radiotherapy to metastatic sites only before entry into the study.

Table I. **Patient characteristics.**

	number of patients.
Sex	
Male	26
Female	4
Median age (range) years	54 (40-69)
Stage	
III	18
IV	12
ECOG performance score	
0	1
1	15
2	10
3	4
Previous treatment	
None	19
Radiotherapy	11

Carboplatin 400 mg/m² was dissolved in 250 ml glucose 5% and administered as a 30 min intravenous infusion on day 1. Vincristine 2 mg was given by intravenous bolus injection on day 1 and 8. Courses were repeated every 4 weeks until disease progression for a maximum of 5 cycles. Dose adjustments were made for myelosuppression (carboplatin) or neurotoxicity (vincristine).

Toxicity and response were measured according to WHO criteria (11) on day 21 after the start of every course. A complete response (CR) was defined as disappearance of all measurable and evaluable lesions; a partial response (PR) was a >50% reduction in the product of the greatest tumor diameter and its perpendicular for all measurable lesions. The term stable disease (SD) was applied if there was a <50% decrease in measu-

rable disease, or a <25% increase of tumor size. Progressive disease (PD) was defined as an increase by >25% in the product of perpendicular diameters of measurable lesions or the occurence of any new lesions. Survival was measured from the start of cytotoxic treatment. Patients were considered evaluable for survival if they completed at least one cycle of chemotherapy.

RESULTS

Response and survival (table II)
One patient died due to aplasia related sepsis (TD) leaving 29 patients evaluable for response. All patients had measurable or evaluable tumor lesions. No CR was seen. Five patients achieved a PR. One of these patients (stage III disease) received radiotherapy to the primary tumor and the mediastinum (50 Gy) while in PR after the third course. Thereafter he received 2 additional courses of carboplatin and vincristine. Two patients had a minimal response, defined by marked clinical improvement without fulfilling criteria for PR. Thirteen patients had disease stabilization, with a median time to progression of 5 months. Six patients had disease progression after one course of carboplatin and vincristine and three patients died during the first course due to disease progression.

For survival analysis the patient who had TD was excluded. One patient, who received concomitant radiotherapy is still alive 27 months after entry into the study. Of all but three of the remaining patients the dates of death are recorded. Median survival for the whole group of patients is 6 months (range 1-27+ months). For patients classified as stage III disease median survival was 7 (range 2-27+) months, for those with stage IV disease this was 4 (range 1-10) months.

Table II.   Response and survival.

Response	number of patients	(%)
Complete response	0	(0)
Partial response	5	(15)
Minimal response	2	(7)
Stable disease	13	(48)
Progressive disease	6	(20)
Early death	3	(10)
Survival (months range)		
All patients	6 (1-27+)	
Stage III disease	7 (2-27+)	
Stage IV disease	4 (1-10)	

Toxicity
The total number of courses was 84, median 2. Trombocytopenia and leukocytopenia WHO grade III/IV was observed in 4 courses only (4.8%). One patient died of aplasia related septicemia during the first course. There were no dose reductions for carboplatin. Ten patients received red blood cell transfusions in the course of their treatment. Peripheral neuropathy presumably due to vincristine was seen in 15 patients (50%). In two of these patients vincristine administration was discontinued because of this toxicity, in the remaining 13 patients the dose of vincristine was reduced to 50%, in 6 patients after the second course and in 7 patients after the third or fourth course. Despite routine antie-

metic drug administration nausea and vomiting WHO grade II/III was encountered in 20 patients (63%). There were no signs of renal or hepatic toxicities. None of the patients complained of hearing loss.

DISCUSSION

Bunn reviewed the activity of single agent carboplatin (doses up to 400 mg/m^2) in NSCLC (12). In 12 studies including both treated and untreated patients the overall response rate was found to be 7% with 1% CR's. In most studies the median survival was not stated. Of particular interest is the recent report of the ECOG (13) who conducted a prospective randomized trial of multidrug regimen (all containing cisplatin) versus single agent carboplatin and iproplatin. Although the response rate in the group of patients treated with single agent carboplatin was only 9% (compared to 20% in the group of patients treated with cisplatin base chemotherapy), median survival was the longest (31 weeks) in patients treated with carboplatin only. Single agent vincristine for NSCLC has been evaluated in two studies by Brugarolas et al. (14,15) The cumulative data from these two studies results in a response rate of 12% among 65 previously untreated patients. There are no data available on the activity of the combination of these two drugs in NSCLC. In the study under discussion in 5 out of 29 evaluable patients (16.7%) a PR was observed, while no CR's were seen. In view of the alleged role of cisplatin as sensitizing agent with radiotherapy (16), the longstanding (27+ months) response achieved in the patient who received radiotherapy between the third and fourth course is worth mentioning. Median survival for the whole group of patients was 6 months, slightly favouring patients with stage III disease (7 months) versus those with stage IV disease (4 months). This difference was not significant. The survival obtained with the combination of carboplatin and vincristine is comparable to that obtained with cisplatin containing chemotherapy regimen. However, toxicity with this combination is low. There was 1 toxic death and WHO grade III/IV myelosuppression was encountered in 4 out of 84 courses (4.8%). In a previous report of carboplatin and vincristine using the same dose schedule in pretreated patients with SCLC this figure was 26% (17). Non haematologic toxicities were encountered more frequently compared to the latter study. Both peripheral neuropathy (50%) and gastro-intestinal symptoms (63%) were a common complication. In conclusion, this study shows that the combination of carboplatin and vincristine is a regimen with a very modest activity in NSCLC. Response rate and median survival are comparable to results obtained with various multidrug regimen in this malignancy. Myelosuppression had a surprisingly low incidence, while non haematological toxicities especially peripheral neuropathy and nausea and vomiting were common. The role of both carboplatin and vincristine in the management of metastatic NSCLC remains to be defined.

REFERENCES

1 Minna JD, Pass H, Glatstein E, Ihde DC. Cancer of the lung. In: DeVita VT, Hellman S, Rosenberg SA (eds). Cancer, principles and practice of oncology 3ed, J.B. Lippincott Co., Philadelphia, 1989, pp 659-664.

2. Williams CJ, Woods R, Levi J, Page J. Chemotherapy for non-small cell lung cancer: a randomized trial of cisplatin/vindesine vs no chemotherapy. Seminars in Oncology 15:58-61, 1988 (suppl 7).

3. Ganz PA, Figlin RA, Haskell CM, La Soto N, Siau J. Supportive care versus supportive care and combination chemotherapy in metastatic non-small cell lung cancer. Does chemotherapy make a difference? Cancer 63:1271-1278, 1989.

4. Rapp E, Pater JL, Willan A. Chemotherapy can prolong survival in patients with advanced non-small cell lung cancer-Report of a canadian multicenter randomized trial. J. Clin. Oncol. 6:633-641, 1988.

5. Muggia FM. Overview of carboplatin: replacing, complementing and extending the therapeutic horizons of cisplatin. Sem. Oncol. 16:7-13, 1989 (suppl 5).

6. Lelieveld P, van der Vijgh WJF, Veldhuizen RW. Preclinical studies on toxicity, antitumour activity and pharmacokinetics of cisplatin and three recently developed derivates. Eur J Cancer Clin Oncol 20:1087-1091.

7. Calvert AH, Harland SJ, Newell DR, Siddik ZH, Jones AC, McElwain TJ, Raju S, Wiltshaw E, Smith IE, Baker JM, Peckham MJ, Harrap KR, Early clinical studies with cis-Diammine-1, 1-Cyclobutane Dicarboxylate Platinum II. Cancer Chemother. Pharmacol. 9:140-147, 1982.

8. Van Echo DA, Egorin MJ, Aisner J: The pharmacology of carboplatin. Sem Oncol 16:1-6, 1989 (suppl 5).

9. Harrap KR, Jones M, Wilkinson CR, Clinck HM, Sparrow S, Mitchley BCV, Clarke S, Veasey A. Antitumor, toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Cisplatin current status and new developments. Prestayko AW, Crooke ST, Carter SK (eds). Academic Press: London, 1980: pp 193-212.

10. Kris M, Cohen E, Gralla R. An analysis of 134 phase II trials in non small cell lung cancer (NSCLC). Proc Fourth World Conf Lung Cancer, Toronto, 1985 (abstr).

11. WHO Handbook for reporting results of cancer treatment. WHO Offset Publication no.48, Nijhoff Den Haag, 1978.

12. Bunn PA. Review of therapeutic trials of carboplatin in lung cancer. Sem Oncol 16:27-33, 1989 (suppl 5).

13. Bonomi PD, Finkelstein DM, Ruckdeschel JC, Blum RH, Green MD, Mason B, Hahn R, Tormey DC, Harris J, Comis R, Glick J. Combination chemotherapy versus single agents followed by combination chemotherapy in stage IV non-small-cell lung cancer: a study of the eastern cooperative oncology group. J Clin Oncol 7:1602-1613, 1989.

14. Brugarolas A, Hansen HH, Siegenthaler P. Clinical study of vincristine in adenocarcinoma of the lung. A study of the early clinical-trial group of the EORTC. Eur J Cancer 16:1643-1644, 1980.

15. Brugarolas A, Lagave AJ, Ribas A, Miralles MTG. Vincristine (NSC 67574) in non small cell bronchogenic carcinoma. Results of a phase II clinical study. Eur J Cancer 14:501-505, 1978.

16. Alvarez V, Cobreros G, Heras A, Lopez Zumel C. Studies on cis-diammineplatinum(II) as a radiosensitizer. Br J Cancer 37:68-72, 1978 (suppl III).

17. Smit EF, Berendsen HH, de Vries EGE, Mulder NH, Postmus PE. A phase II study of carboplatin and vincristine in previously treated patients with small cell lung cancer. Cancer Chemother Pharmacol 25:202-204, 1989.

Chapter 8

CONTINUOUS INFUSION OF CARBOPLATIN ON A 21 DAY SCHEDULE - A PHASE I AND PHARMACOKINETIC STUDY

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SUMMARY

A phase I study with continuous infusion of carboplatin for 21 days every 6 weeks using a venous access port and portable pump was performed over a dose range of 12-32 mg/m²/day, with increments of 2 mg/m²/day. Forty-four patients received 107 courses (median 2, range 1-9). WHO grade III/IV leukopenia and thrombocytopenia occurred in 1 out of 7 pts at 30 mg/m²/day, and in 2 out of 6 and 4 out of 6 patients at 32 mg/m²/day respectively. Cumulative platelet depression was found at dose levels of 28 mg/m²/day or more. Median glomerular filtration rate (GFR) and effective renal plasma flow, monitored by radioisotope clearances at doses ≥20 mg/m²/day, decreased 8.2% (p < 0.05) and 10.9% (p < 0.01) after 2 courses. There was a relationship (r=0.50, p < 0.0002) between percent platelet depression and GFR. No other toxicity was observed. Of the 17 patients evaluable, one complete response, four partial responses were observed. In addition, six patients had stable disease. Pharmacokinetic analysis of total and ultrafiltrable platinum (UFPt) was performed by atomic absorption spectrophotometry. Steady state plasma levels for UFPt were reached after 8 hours. These levels could be detected from the 20 mg/m²/day dose. During steady state, carboplatin dose and UFPt plasma levels were not correlated, but steady state UFPt and GFR (r=-0.27, p < 0.05) were. Twenty-four percent of total Pt was present as UFPt during steady state (x=160±10 µg/l). Total body clearance of UFPt exceeded GFR 2.2 times. Mean area under the curve for UFPt during continuous infusion was 4921.8±301.72 mg.min/l. For total Pt steady state plasma levels were not reached: total Pt plasma levels increased between day 7 and day 21 (p < 0.0001). There was a significant relation between total Pt serum levels day 7, 14 and 21 and the drug dose administered. Immunohistochemical analysis of DNA bound Pt in leukocytes showed a linear increase from day 7 to day 14 to day 21 (r=0.97) between DNA bound Pt and duration of infusion in individual patients. The maximum tolerable dose of carboplatin is 30 mg/m²/day for 21 days (total dose 630 mg/m²) and is recommended for phase II studies.

INTRODUCTION

One of the reasons for the inability of chemotherapeutic regimes to produce substantial cure rates in solid tumors is thought to be ineffective drug dosing or scheduling (1, 2). One way to overcome this problem, may be the administration of chemotherapy by continuous infusion, which has been under extensive study in the recent years. The rationale to undertake such studies is provided by cytokinetic and pharmacologic considerations (3, 4). The cisplatin analogue carboplatin seems to be a good candidate for prolonged continuous infusion. In vitro data suggest for carboplatin (5, 6) as well as for cisplatin (5, 7-9) that prolonged low dose exposure does increase cell kill. Once bifunctionally bound to DNA, the mode of action of carboplatin is not different from cisplatin (10), but the platinum (Pt) induced DNA lesions (DNA interstrand cross-links) appear later (6-12 hours) after incubation of cell suspensions with carboplatin compared to cisplatin (11). Several factors, such as the lower chemical reactivity of carboplatin compared to cisplatin and altered intracellular handling of the drug may account for this phenomenon, but a decreased or slower uptake of carboplatin could also play a role. In addition, adduct formation may be a cell cycle specific phenomenon (12). As non protein bound Pt, ultrafiltrable Pt (UFPt), is especially responsible for the cytotoxic DNA lesion of carboplatin (13, 14), cytotoxicity may be enhanced by expanding the exposure duration of cells to UFPt. Currently no evidence suggests that any schedule of carboplatin has a superior therapeutic index (15). Cisplatin toxicity can be modified by different methods of administration. Compared to bolus injection continuous infusion schedules produce substantially less nephro- and neurotoxicity, even with dose increment (16-21). The purposes of this study were to determine the maximum tolerable dose of carboplatin in a 21-day continuous infusion schedule and to characterize clinical toxicity. Plasma pharmacokinetic studies were performed and Pt-DNA adduct levels were measured in leukocytes of patients during continuous infusion.

MATERIALS AND METHODS

Patients and chemotherapy

Forty-four patients were entered in the study between July 1987 and August 1989. All patients had histologically proven advanced cancers and were considered refractory or resistant to standard therapies. In addition all patients met the following criteria: (1) Age from 21 to 75 years. (2) Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 3. (3) estimated life expectancy of ≥ 2 months (4) serum creatinine level ≤ 1.45 mg/dl and/or glomerular filtration rate (GFR) ≥ 60 ml/min (see below) (5) serum bilirubin level ≤ 2.0 mg/dl (6) leukocyte count $\geq 3 \times 10^9$/l (7) platelets $\geq 100 \times 10^9$/l. No patient had received antineoplastic treatment during the 3 weeks before entry. Informed consent was obtained from all patients. The study was approved by the Medical Ethical Committee of the University Hospital Groningen, The Netherlands. At the time of entry the following studies were per-

formed in all patients: full physical examination, complete blood cell counts, electrolytes, liver and renal function, chest X-ray and ECG.

For continuous infusion an implanted venous access port (Infuse-A-Port™) and a portable pump (Graseby Medical MS 16a™) were used. A 20 ml Luer Lock syringe with carboplatin (Bristol Myers, Weesp, The Netherlands) was connected to the port via an extension tube and a Huber point needle. The syringe was replaced with freshly constituted carboplatin solution every 48 hours. Patients formulated and reconstituted the drug at home (22). With suitable HPLC methods (23) we proved that the carboplatin stock solution (10 mg/ml 5% glucose) was stable for at least 4 weeks at 4⁰C and that the carboplatin for continuous infusion (stock solution diluted with 5% glucose to a volume of 17 ml) was stable for at least three days at room temperature. The whole treatment was performed on an outpatient basis (24).

The study was started at a dose of 12 mg/m^2/day for 21 days, since this is equivalent to 250 mg/m^2, a dose which has no reported major toxicity when given as a bolus (25). Courses were repeated after a three week therapy free interval if no disease progression was evident and no unacceptable toxicity was encountered. The dose was escalated with 2 mg/m^2/day if no unacceptable toxicity was seen in any of three patients treated at that dose level. Unacceptable toxicity, using the World Health Organization (WHO) grading system (26) was defined as any parameter reaching grade III toxicity, except neurotoxicity (limit grade II), while any degree of alopecia was considered acceptable. Doses were not escalated in the individual patient. During each 21 day course, complete blood cell count, electrolytes, liver and renal function were measured weekly. During the three week therapy free interval toxicity was evaluated at least on days 37 and 42 since the start of chemotherapy. On days 0 and 42 of each cycle ECG and chest X-rays were performed.

Starting at the 20 mg/m^2/day dose level GFR and effective renal plasma flow (ERPF) were studied at time of entry into the study and between days 7 and 14 after each course. GFR and ERPF were measured simultaneously in supine position with radioisotopes. ERPF was determined by measuring the clearance of ^{131}I-hippuran and GFR by the clearance of ^{125}I-iothalamate. After a standard primary dose and sustaining infusion for two hours, two hour clearances were determined. Values are the mean of two 2 hours clearances (27).

To be evaluable for response, the patient with measurable disease had to be followed for at least 6 weeks since the start of treatment. Tumor measurements were obtained at entry and after two treatment cycles, or when there was evidence of disease progression after or during a course of carboplatin. Complete response (CR) indicated the documented disappearance of all signs and symptoms of detectable tumor and no development of new lesions. A partial response (PR) was defined as a decrease of at least 50% in the sum of the products of the two largest perpendicular diameters of all measurable lesions and no concomitant occurrence of new lesions. The situation in which no change or decrease of less than 50% of the sum of the products of the two largest perpendicular diameters of measurable lesions occurred was defined as stable disease. Disease progression was defined as a 25% increase in the aforementioned lesions.

Sampling procedure

At every weekly outpatient visit blood specimen were obtained. At the 30 mg/m²/day dose level, additional samples (1,2,3,4,5,6,8,12,24,36,48 and 72 hours) were taken in three patients during the first infusion days as well as after completion of the first cycle (1,2,3,4,5,6,8,12,24,36,48,72 hours). All samples were collected in heparinized tubes (Venoject®) on ice. Samples were centrifuged (1500 g for 5 min at ambient temperature) and 2 ml of plasma were ultrafiltrated (4470 g for 60 min at ambient temperature) with an Amicon Centifree TM micropartition system provided with YMT membranes (Amicon, Oosterhout, The Netherlands) in order to generate UFPt samples. The plasma and plasma ultrafiltrate were stored at -20°C to await analysis.

From duplicate blood samples obtained at the same time points as described above white blood cell suspensions were generated with lysis of erythrocytes with ammonium chloride on ice, according to previously published techniques (28, 29).

Analytical procedure

Pt concentrations in plasma (total Pt) and UFPt were determined by flameless atomic absorption spectrometry (AAS) (30). The amount of Pt was determined with a model 1275 atomic absorption spectrophotometer with background correction using a Deuterium lamp and a GTA-95 graphite tube atomizer and an autosampler. Plasma samples for determination of total Pt were analyzed after 3:1 dilution in a 1% solution of 23 Lauryl Ether 30% in order to avoid matrix effects. A callibration curve was made in the same organic matrix. Because we expected the UFPt plasma levels to be below the lower detection limit of 100 µg/l by this method, we devised an extraction method for UFPt. In short, Pt was extracted from the ultrafiltrate by adding 1 ml potassium iodide (1 Mol/l), 2ml methanol, 5ml dithizone reagent (0.2mMol diphenylthiocarbazone/L chloroform) and 1 ml sulfuric acid 50% to 1 ml ultrafiltrate. All chemicals were of reagent grade. Samples were vortexed for 10 sec, shaken for 20 min in a shaking apparatus and centrifuged for 5 min at 1500 g; the organic phase was collected and dried under nitrogen at 50°C. The Pt containing residue was thereafter dissolved in 200 µl dimethylformamide. The amount of Pt was determined as described above. By this method Pt from carboplatin is extracted from ultrafiltrates for 100% (Coefficient of variation 5% at 0.5 mg/l fetal calf serum). The lower detection limit for UFPt is 2 µg/l.

Quantitation of Pt-DNA adduct formation

From the isolated leukocyte suspensions cytospin preparations were made. Pt-DNA adducts were visualized and quantitated by an immunocytochemical method developed by Terheggen et al. with some minor modifications (31).

Pharmacokinetic and statistical analysis

For postinfusion pharmacokinetics, the plasma concentrations of UFPt and total Pt from each patient were subjected to pharmacokinetic analysis using a computer analysis program (32). The program includes a correction for infusion time (33), and a statistical ana-

lysis comparing the accuracy of models containing different numbers of phases (34). For plasma area under the curve ($0 \rightarrow \infty$ min) (AUC) determination during continuous infusion, a linear relationship was found to show the best fit between time and drug level before steady state was reached in the three patients treated at 30 mg/m²/day. Their AUC for UFPt was calculated using the trapezoidal rule. A three compartment model was found the best fitting model for elimination of UFPt and a two compartment model for total Pt post continuous infusion. For AUC for UFPt calculation in the patients for whom 4 data points (t = 0, 7, 14 and 21 days) were available two assumptions were made. First, the time to reach steady state plasma levels (8 ± 0.2 hours, see results section) was extrapolated from the three patients mentioned above to the whole group of patients. Secondly, for calculation of the part of the AUC of UFPt in the post continuous infusion phase, which represents less than 1% of the total curve, elimination half lives found in the three patients of whom a complete curve was obtained were used.

Statistical significance was determined with the Student's t-test for the difference between the mean of two samples, Wilcoxson's test was applied to renal function parameters. Values of $p \geq 0.05$ were considered not significant.

RESULTS

The clinical characteristics of the patients entered in this protocol are summarized in Table I. Two patients had received previous cisplatin containing chemotherapy. The 44 patients were treated with one to nine courses (median two) for a total of 107 courses. All patients completed at least one course of carboplatin and all but one patient are fully evaluable for toxicity.

Toxicity
Myelosuppression was dose limiting in this schedule. Nadir counts of leukocytes and platelets occurred between days 28 and 40, platelet nadirs preceded leukocyte nadirs for about one week. In most patients recovery had occurred at day 42 of each cycle to allow for a second course. Twelve courses (11%, eight patients) had to be postponed for a median of seven days because of insufficient bone marrow recovery. Up to a dose level of 30 mg/m²/day no unacceptable toxicity was seen during the first cycle, except for one patient (leukocytopenia WHO grade III) treated with 24 mg/m²/day who had a documented intercurrent viral infection. In addition, 2 patients, treated with 16 mg/m²/day and 20 mg/m²/day had leukocytopenia WHO grade III during their third cycles. Details of haematological toxicity for doses 28-32 mg/m²/day are summarized in Tables II and III. At 30 mg/m²/day leuko- and thrombocytopenia grade III/IV occurred in one out of seven patients (15 courses). In three out of six patients entered at the 32 mg/m²/day dose grade III/IV haematological toxicity was encountered in the first course and therefore the study was finished at this dose level. One of these patients had trombocyte count of 18x10⁹/l at day 28, and leukocyte count of 0.7x10⁹/l at day 34. Because of tumor progression no furt-

Table I. **Clinical characteristics of patients treated with continuous infusion carboplatin (n=44).**

	number of patients
Median age (range)	57 (21-69) years
Sex	
Male	30
Female	14
ECOG performance status	
0	9
1	11
2	13
3	11
Primary site	
Esophageal/Gastric carcinoma	10
Bronchial carcinoma	10
Genito-urinary carcinoma	9
Adenocarcinoma of unknown primary	7
Pancreatic carcinoma	3
Colon carcinoma	2
Soft tissue sarcomas	3
Previous treatment	
None	22
Chemotherapy	13
Radiotherapy	2
Radiotherapy + Chemotherapy	7

her outpatient visits were performed. The patient died under unknown circumstances at home day 37. In the three additional patients treated at this dose level no haematological toxicity was seen during their first course. However, two of these three patients experienced grade IV thrombocytopenia in their second respectively third course of carboplatin. None of the patients had to be hospitalized for neutropenia associated infection. Prophylactic platelet transfusions were administered to four out of the six patients described above, none had symptomatic bleeding episodes. Eight patients, treated at various dose levels required red blood cell transfusions. Evidence of cumulative bone marrow suppression was seen after two cycles in patients receiving carboplatin 28 mg/m²/day or more. At these doses there was a significant ($p < 0.05$, Wilcoxson's test for paired samples) difference in percent reduction in rombocyte count after the second course as compared to the first course (n=17), indicating cumulative bone marrow toxicity. No such differences could be found for leukocyte counts or haemoglobin. A relationship between percent platelet depression induced by carboplatin and GFR ($r=0.50$, $p < 0.0002$) (fig.1) could be established, but not with AUC's for UFPt ($r=0.22$, $p > 0.5$).

**Table II.** **Haematological toxicity: No. courses associated with WHO toxicity grade:**

Dose	no pts.	no courses	1	2	3	4	1	2	3	4	1	2	3	4
			leukocytes				thrombocytes				haemoglobin			
28 mg/m²	3	8	2	1	0	0	1	4	0	0	3	0	0	0
30 mg/m²	7	15	8	1	0	0	2	3	0	1	5	0	0	0
32 mg/m²	6	14	0	3	1	1	0	1	3	3	5	2	0	0

Table III. Haematological toxicity: Median nadir counts (range) x 109/L.

	course 1		course 2		course 3	
Dose	leukocytes	thrombocytes	leukocytes	thrombocytes	leukocytes	thrombocytes
28 mg/m²	3.4 (2.6-7.3)	122 (119-130)	3.8, 4.8	54, 72	4.5	72
30 mg/m²	3.0 (2.2-6.9)	187 (9-376)	3.7 (3.2-5.7)	155 (60-320)	3.5, 5.7	161, 369
32 mg/m²	2.4 (0.7-6.8)	42 (12-223)	5.6 (5.0-14.8)	122 (30-229)	3.4 (2.2-4.0)	49 (20-118)

No other major toxicity was found. In particular, there was no hepatic, renal toxicity (as measured by the WHO grading system, but see also below), neuro- or ototoxicity. Mild nausea (grade I) occurred in 14 patients and responded to simple anti-emetics, for instance oral metoclopramide 30 mg/day. Partial alopecia was seen in one patient (24 mg/m²/day). All patients treated with 20 mg/m²/day or more (n=28) were evaluable for renal function assessed by a radiochemical method. Following the first course of carboplatin there were no significant changes in GFR and ERPF. After two courses, or a
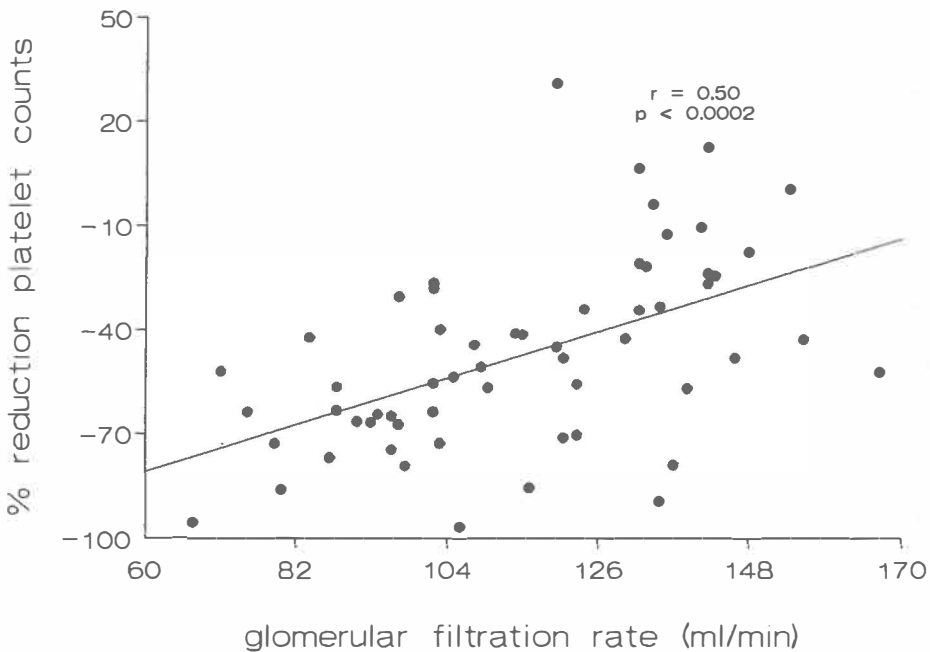
Figure 1. The relationship between glomerular filtration rate (ml/min) and percent reduction in platelet counts. Each point represents an individual patient.

cumulative dose of 840-1344 mg/m², there was a significant fall in both GFR (median - 8.2%, range -5.0 - -21.9%, p<0.05) and ERPF (median 10.9%, range -7.2 - -29.6%, p < 0.01). Within this dose range no dose related renal toxicity could be demonstrated. The small number of patients receiving three or more courses did not allow for further analysis of renal function parameters.

Regarding catheter complications, vena cava superior syndrome was seen in two patients. In one patient treatment had to be discontinued because of a obstruction of the brachiocephalic vein. Temporary catheter occlusion, for a maximum of 32 hours, was seen in 11% (n=12) of the courses. Although the patients prepared the carboplatin solution for infusion at home themselves, septicemia was not observed.

Tumor response

Out of the 17 patients who were evaluable for response, five responses (1 CR, 4 PR) were noticed, including a PR in one patient with ovarian cancer. The latter patient had previously received a total cumulative dose of 600 mg/m² cisplatin and obtained a disease free interval of 18 months. The five responding patients are summarized in Table IV. In addition, six patients had stable disease with a median time to progression of four months. Six patients with measurable disease had disease progression after one course.

Table IV. Tumor response (no evaluable patients = 17).

Primary site	Carboplatin dose	Response	Response duration
Lung (squamous)	16 mg/m²/day	PR	5 months
Ovary	18 mg/m²/day	PR	3 months
Cervix (adeno)	20 mg/m²/day	CR	6 months
Lung (adeno)	30 mg/m²/day	PR	3.5 months
Carcinoma of the papilla of Vater	32 mg/m²/day	PR	6$^+$ months

Pharmacokinetics

All treatment courses were evaluable for pharmacokinetic analysis in regard to total Pt. UFPt pharmacokinetics could be analyzed in 60 courses (27 patients) starting from the 20 mg/m²/day dose. During continuous infusion of carboplatin at 30 mg/m²/day both total Pt and UFPt became detectable after 2 hours. A linear increase in UFPt was seen until after 8 ± 0.2 hours steady state concentrations for UFPt were reached. Up to 24 hours after start of infusion an average of 85% of total Pt was present as UFPt (n=3). Thereafter a gradual increase was seen in the relative amount of protein bound Pt, reflected by the decreasing percentage UFPt at days 7 (29.6±2.10%) (x±SE), 14 (21.6±2.00%) and 21 20.8±1.71%) for the whole group of patients (n=27). During the infusion period a mean of 24.5%±1.71% of total Pt was present as UFPt (n=27), but there was considerable inter- and intrapatient variability. The mean UFPt plasma concentration during steady state was 160±10 µg/l (n=27). During steady state there was no significant relationship between
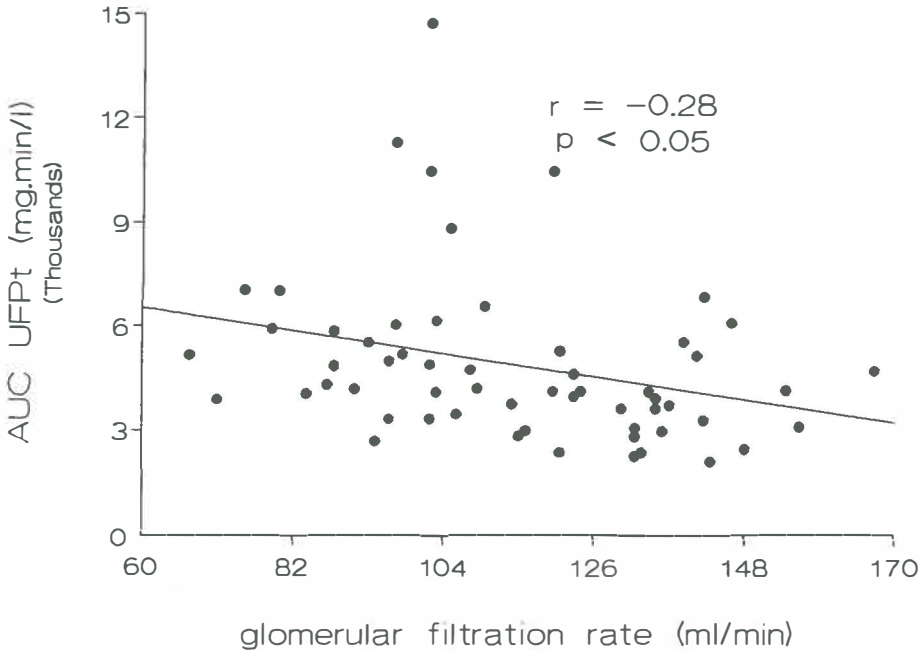
Figure 2. The relationship between glomerular filtration rate (ml/min) and area under the time versus concentration curve of ultrafiltrable platinum (mg.min/l). Each point represents an individual patient.
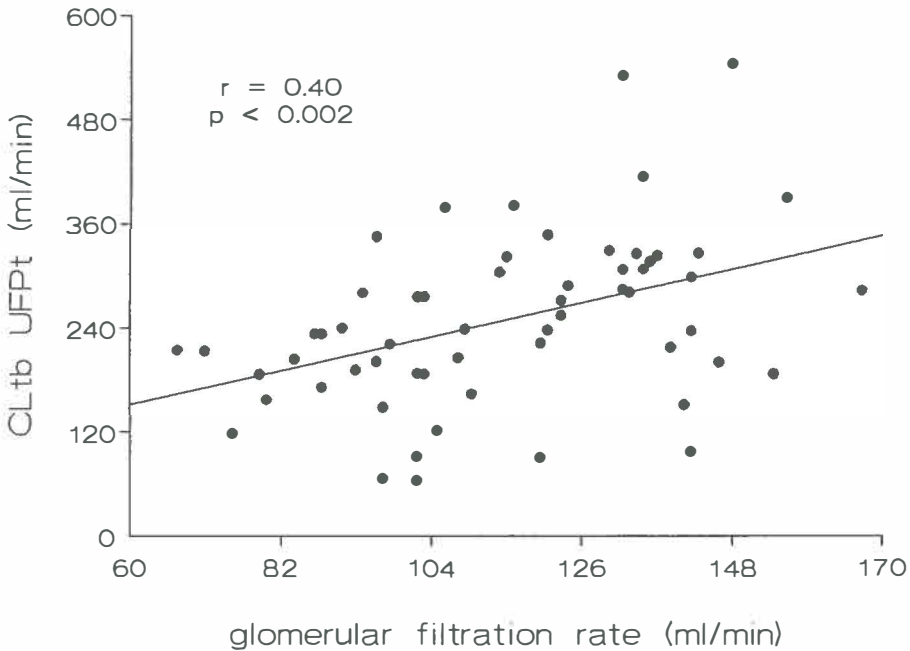
Figure 3. The relationship between glomerular filtration rate (ml/min) and total body clearance of ultrafiltrable platinum (ml/min). Each point represents an individual patient.

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dose and mean UFPt plasma level (r=0.17, p > 0.19). The plasma AUC of UFPt was also dose independent in the dose range studied; the mean AUC of UFPt was 4921.8±301.72 mg.min/l (n=60). During subsequent courses plasma AUC of UFPt did not differ significantly from the first course in the 17 patients receiving two courses or more. A weak relationship (r=-0.28, p < 0.05) could be established between AUC of UFPt and GFR (fig.2).

Assuming linear pharmacokinetics, total body clearance (CL_{tb}) (dose/AUC) and GFR were correlated (r=0.40, p < 0.002) (fig.3). The mean (SE) of CL_{tb} for UFPt was 248.3 (13.01) ml/min; approximately 2.2 times the GFR (mean (SE) 115 (2.92)). After cessation of therapy (30 mg/m²/day) plasma UFPt levels were detectable for 24 hours and showed a triexponential decay with $t1/2\alpha$ of 43.5 min., $t1/2\beta$ of 111.3 min. and $t1/2\gamma$ of 1382.7 min (n=3).
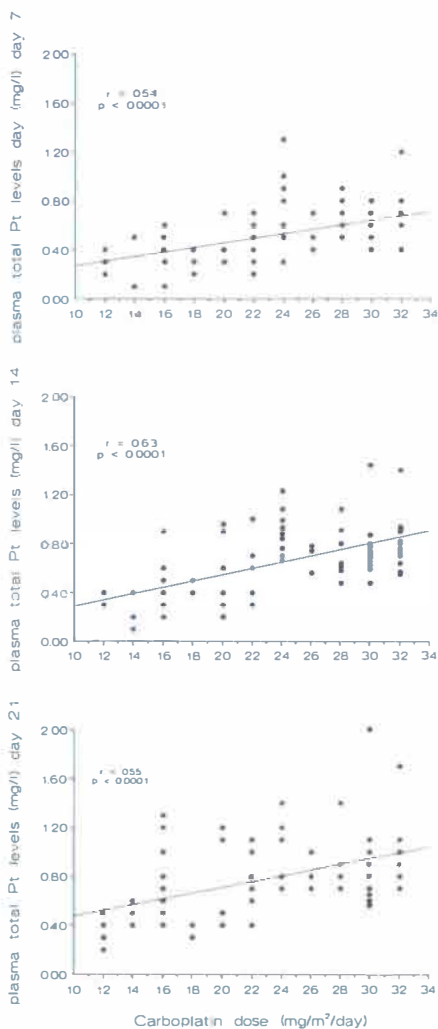
Figure 4. The relationship between carboplatin dose administered (mg/m²/day) and plasma total platinum levels (mg/l) day 7 (top), day 14 (middle) and day 21 (bottom). Each point represents an individual patient.

For protein bound Pt steady state plasma concentrations were not reached during the infusion period. There was a significant increase (p < 0.0001) in total Pt plasma levels between days 7 and 21. Postinfusion plasma levels of total Pt showed a biexponential decay, with terminal half life of > 7 days (n=3). Total Pt could be detected for at least 21 days post infusion, accumulation of protein bound Pt was found in all patients receiving more than one course. In contrast to UFPt plasma levels, there was a significant relationship between total Pt plasma levels days 7, 14 and 21 and the dose administered (fig.4). Total Pt plasma levels (first courses) ranged from 100- 800 µg/l (day 7), 100-1080 µg/l (day 14) and 300-1400 µg/l (day 21). There was a significant negative correlation between GFR and total plasma Pt concentration at day 21 (end of infusion) (r=-0.45, p < 0.002) (fig.5).
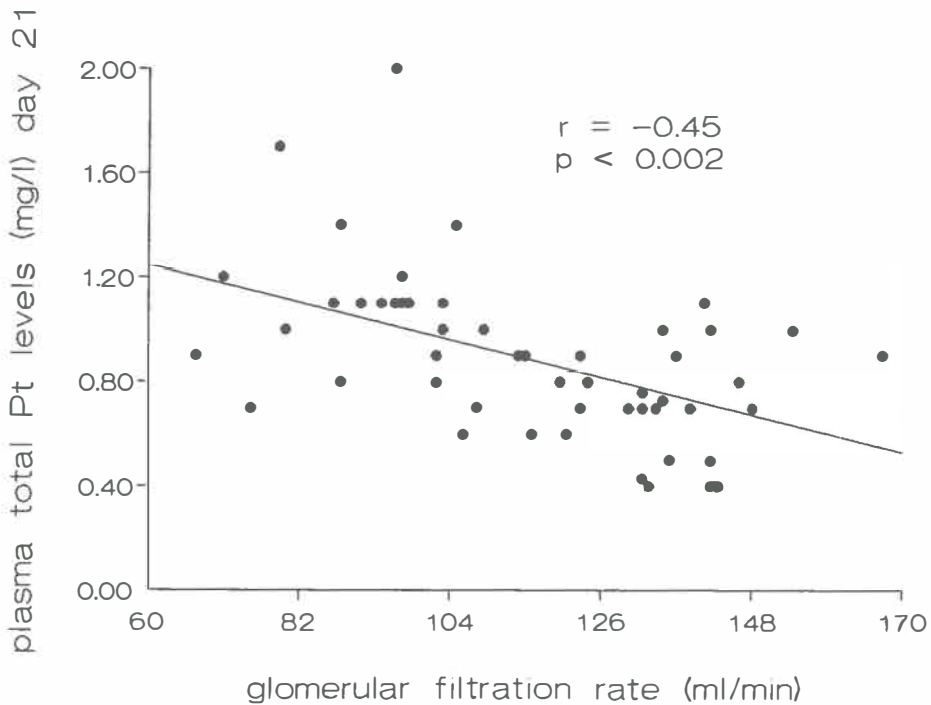

Figure 5. The relationship between glomerular filtration rate (ml/min) and plasma total platinum levels day 21 (mg/l). Each point represents an individual patient.

Quantitation of Pt-DNA adduct formation

Figure 6 shows the relationship between nuclear staining density, which reflects Pt-DNA binding, and dose of carboplatin administered in five patients. There was a linear relationship (r=0.97) between dose and nuclear staining density on day 7, day 14 and day 21 for individual patients. No evidence of saturation of Pt-DNA adduct formation or of an

equilibrium between DNA repair processes and adduct formation (as expressed by steady state levels of the latter) was found. There was no correlation between dose administered or AUC for UFPt and nuclear staining density. In fact, the patient with the highest AUC for UFPt had the lowest adduct level throughout the infusion period.
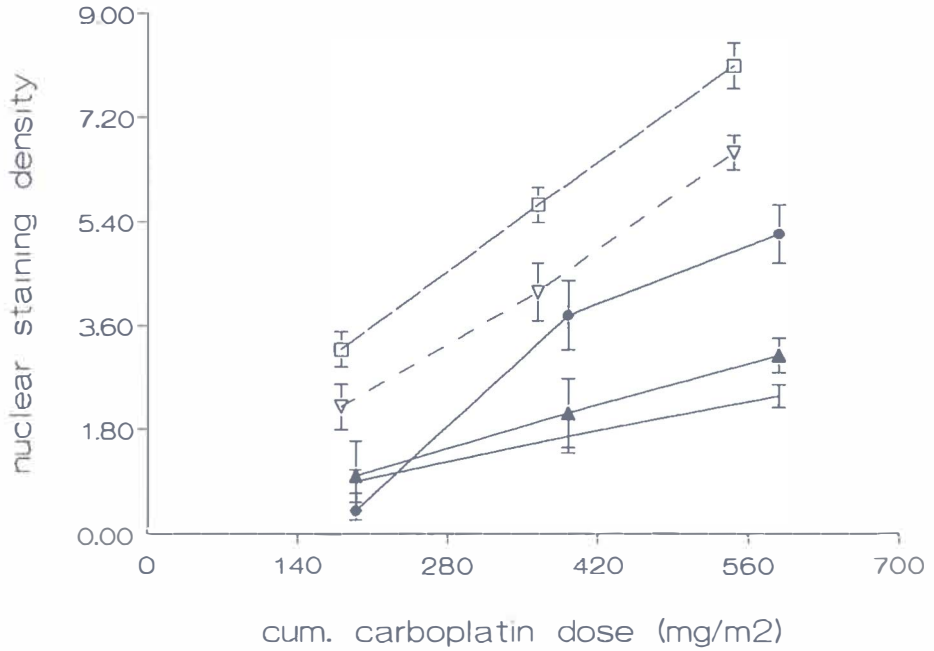
Figure 6. The relationship between the carboplatin dose administered (mg/m^2) and platinum-DNA adduct level (Arbitrary Units) in leukocytes. Each point represents the nuclear staining density $\pm$ SE, in a single patient, as measured by microdensitometry in 20-30 nuclei from two cytospin slides.

DISCUSSION

Carboplatin has been evaluated in phase I studies in several schedules. The most frequently studied schedule is a single dose repeated every four to five weeks. Also 24 hour continuous infusion (35), daily times five (36, 37) and weekly times four bolus (38) schedules were investigated. In this phase I study patients were treated continuously during 21 days with carboplatin. Dose limiting thrombocytopenia and leukopenia occurred at 32 mg/m^2/day (cumulative dose 672 mg/m^2). At 30 mg/m^2/day (cumulative dose 630 mg/m^2), grade III/IV myelosuppression was seen in one out of six patients. Nadirs of platelets and leukocytes tended to occur at the end of the three week treatment free interval. Thus, courses cannot be repeated sooner than every six weeks. In individual patients cumulative myelosuppression occurred from carboplatin doses of 28 mg/m^2/day or more.

It should be stressed that at 30 mg/m²/day, nausea did not exceed grade I and vomiting was not encountered. A similar dose of carboplatin (600 mg/m²) given as a single bolus (35, 39) or daily times five scheme (625 mg/m²) (36) produces grade III/IV thrombocytopenia in 100%, and severe gastrointestinal toxicity (grade III/IV) in the majority of them. The recommended dose of carboplatin given as a single bolus infusion is 400-450 mg/m², for good risk patients (15). At this dose level thrombocytopenia grade III/IV occurs in 0-75% of patients, and nausea and vomiting grade II or more in > 40% of patients (40-49). Thus, with continuous infusion it is possible to deliver a higher total dose of carboplatin with less toxicity compared to a single bolus infusion. A similar phenomenon was noticed by Rozencweig et al (36) and Van Echo et al (37) investigating daily times five bolus infusion. From the point of view of dose intensity, it is apparent that with continuous infusion it is possible to deliver the same dose intensity, e.g. 100 mg/m² carboplatin/week, as with bolus infusion schedules, but with less toxicity as pointed out above.

Special attention was paid to renal function impairment. Using the WHO grading system, there was no evidence of renal toxicity. In a previous report (50) we found, after a cumulative dose of 800 mg/m² of carboplatin given as a bolus infusion (10 patients), a significant fall in both GFR (median 19%) and ERPF (median 14%) as measured by radioisotope clearances. Notably, there was no fall in endogenous creatinine clearances, a finding confirmed by others measuring renal function impairment after cisplatin containing regimen (51, 52). In the present study a less pronounced but still significant decrease in GFR (median 8.2%) and ERPF (median 10.9%) after two courses (cumulative dose 840-1344 mg/m²) was seen. Although the exact mode of Pt induced renal toxicity remains unknown, one may speculate, analogous to cisplatin, that this toxicity is associated with maximum peak plasma levels of Pt (21). The absence of peak levels of UFPt with continuous infusion could explain the lesser degree of renal toxicity compared to our previous study with bolus carboplatin. We did not perform renal function studies following cessation of treatment. Therefore, we cannot exclude the possibility of recovery of GFR and/or ERPF to baseline levels, as was observed by Hardy and coworkers (53) for GFR (measured by ^{51}CrEDTA clearances) following treatment with high dose carboplatin (1 gr/m²) after a treatment free interval of 3 months.

We were able to establish a significant relationship between percent reduction in platelet counts and GFR. Egorin and coworkers (54) correlated renal function and carboplatin induced thrombocytopenia. Furthermore, Calvert et al. (55) saw predictable hematological toxicity based on target AUC and ^{51}CrEDTA clearances in a prospective study. In contrast, Colombo et al. (41) using the same formula as Egorin et al. (54), found that toxicity of carboplatin in pretreated patients can be quite variable, even after dose adjustments for creatinine clearances.

No data are available concerning pharmacokinetics of continuous infusion of carboplatin in contrast to bolus infusion carboplatin, which has been the subject of several reports (37, 39, 40, 54, 56-62). Plasma steady state levels of UFPt during continuous infusion were reached after 8 hours, that is approximately 4.5 times the calculated post infusion ß half life. The high percentage of UFPt during the first 24 hours of infusion (85%)

reflects the slow protein binding rate of carboplatin. Harland et al. (57) reported an *in vitro* half life of 30 hours for free carboplatin. During steady state aproximately 25% of total Pt was present as UFPt, confirming the low protein binding rate of carboplatin reported by others. In contrast to literature data concerning bolus infusion of carboplatin, the AUC for UFPt did not significantly increase in the dose range studied (420 mg/m^2-672 mg/m^2). However, total Pt plasma levels (dose range studied 252 mg/m^2-672 mg/m^2) did increase with dose, which is in concordance with literature data. Currently, we have no conclusive explanation for the absence of increment of AUC for UFPt with dose. This may be due to the relatively narrow dose range studied and the low serum levels of UFPt in comparison with single bolus administration. In three patients studied for post infusion pharmacokinetics a triexponential decay in plasma levels for UFPt was found, with α and ß half lifes comparable to those reported in the literature (43.5 min and 111.3 min), but also with a long γ half life of more than 20 hours. This third elimination phase may represent the continuous release of Pt from proteins. Alternatively, the discovery of a γ phase may be due to the extraction method used for UFPt, which has a lower detection limit of 2 µg/l. In general, detection limits for Pt measured by AAS are in the range of 100 µg/l, and for HPLC methods 0.5-10 µg/ml. Several authors have found that CL_{tb} for UFPt correlates with GFR (54, 57, 63), but others (61, 58) could not find consistent relationships. In this study the mean CL_{tb} for UFPt was approximately 2.2 times the GFR. This is a consistent finding throughout the literature concerning pharmacokinetics of carboplatin (61). CL_{tb} for UFPt was also correlated with GFR. Given the single measurement of GFR during one six week period, we feel that there is sufficient evidence that pharmacokinetics of carboplatin are not significantly altered by extending the infusion period to 21 days.

Our study is the first study where plasma Pt data and Pt-DNA adduct formation levels are simultaneously obtained. In the five patients who are evaluable for Pt-DNA adduct formation in leukocytes, there was a linear dose response relation in the individual patient. Despite low levels for UFPt in plasma, an ongoing platination of DNA in leukocytes was observed. No steady state levels were reached during the 21 day infusion period. Also, no correlation could be found between UFPt plasma steady state level and nuclear staining density. Because the numbers are small this observation remains to be confirmed in a larger series. In a previous study with a similar continuous infusion schedule with mitoxantrone (64), the amount of drug accumulated in leukocytes also increased significantly during the infusion period, in presence of plasma steady state levels. For antitumor activity the intracellular Pt levels are probably more important than plasma levels. When cultured cell lines, made resistant to cisplatin *in vitro*, are compared to the cell lines they were derived from, the resistant cells generally have lower levels of Pt-DNA binding (65, 66). Reed et al. (67) have shown that Pt-DNA adducts in leukocyte DNA correlate with tumor response in ovarian cancer patients receiving cisplatin or carboplatin based chemotherapy. Patients who had a response on chemotherapy had higher median adduct levels than those who showed no response. In an earlier report by the same group (68) it was shown that cisplatin-DNA adduct levels increased in a dose dependent fashion. The removal of cisplatin induced Pt-DNA adducts in circulating leukocytes appears to set in rapidly after end

of infusion. Fichtinger-Schepman et al (69) observed a decrease of more than 50% in the number of intrastrand DNA cross-links in peripheral leukocytes within the first 24 hours after cisplatin infusion in six patients. In these studies plasma pharmacokinetic data of Pt were not provided.

We may conclude that the optimal dose of carboplatin in a continuous infusion schedule for 21 days is 30 mg/m²/day. Hematological toxicity is clearly reduced compared to bolus administration and non hematological toxicities are less pronounced. Pharmacokinetics of carboplatin are not altered by this method of drug delivery, with a possible exception of a third elimination phase. Pt-DNA adduct formation in leukocytes increased during the whole infusion period. Whether continuous infusion has advantage over bolus infusion in efficacy should be evaluated in prospective trials. The number of responses seen in this phase I study may justify such an approach.

Acknowledgements

REFERENCES

1. Frei III E, Canellos GP: Dose: a critical factor in cancer chemotherapy. Am J Med 69: 585-594, 1980
2. Bonadonna G, Valagussa P: Dose response effect of adjuvant chemotherapy in breast cancer. New Eng J Med 304: 10-15, 1981
3. Vogelzang NJ: Continuous infusion chemotherapy: a critical review. J Clin Oncol 2: 1289-1304, 1984
4. Carlson, RW, Sikic BI: Continuous infusion or bolus injection in cancer chemotherapy. Ann Int Med 99: 823-833, 1983
5. Roed H, Vindelov LL, Christensen IJ et al: The cytotoxic activity of cisplatin, carboplatin and teniposide alone and combined determined on four human small cell lung cancer cell lines by the clonogenic assay. Eur J Cancer Clin Oncol 24: 247-253, 1988
6. Clinical brochure, CBDCA (NSC-241240), Bethesda, MD, National Cancer Institute, 1982
7. Drewinko B, Brown BW, Gottlieb JA: The effect of cis-diamminedichloro-platinum(II) on cultured human lymphoma cells and its therapeutic implications. Cancer Res 33: 3091-3095, 1973
8. Bergerat JP, Barlogie B, Drewinko B: Effects of cis-dichlorodiammine-platinum(II) on human colon carcinoma cells in vitro. Cancer Res 39: 1334-1338, 1979
9. Rupniak HT, Whelan RDH, Hill BT: Concentration and time dependent inter-relationships for antitumor drug cytotoxicities against tumor cells in vitro. Int J Cancer 32: 7-12, 1983
10. Knox RJ, Friedlos F, Lydall DA et al: Mechanism of cytotoxicity of anticancer platinum drugs: evidence that cis-diamminedichloro-platinum(II) and cis-diammine-(1, 1-cyclobutanedicarboxylato)platinum(II) differ only in the kinetics of their interaction with DNA. Cancer Res 46: 1972-1979, 1986

11.   Micetich KC, Barnes D, Erickson LC: A comparative study of the cytotoxicity and DNA-damaging effects if cis-(diammino)(1, 1-cyclobutane-dicarboxylato)-platinum(II) and cis-diamminedichloroplatinum(II) on L1210 cells. Cancer Res 45: 4043-4047, 1985

12.   Rabes HM, DNA adducts and cell cycle. J Cancer Res Clin Oncol 112: 189-195, 1986

13.   Gormly PE, Bull JM, LeRoy AF et al: Kinetics of cis-dichlorodiammine-platinum. Clin Pharmacol Ther 25: 351-357, 1979

14.   Takahashi K, Seki T, Nishikawa K et al: Antitumor activity and toxicity of serum protein-bound platinum formed from cisplatin. Jpn J Cancer Res (Gann) 76: 68-74, 1985

15.   Ozols RF: Optimal dosing with carboplatin. Sem Oncol 16: 14-18, 1989 (Suppl 5)

16.   Lokich JJ: Phase I study of cis-diamminedichloroplatinum(II) administered as a constant 5-day infusion. Cancer Treat Rep 64: 905-908, 1980

17.   Salem P, Khalyl M, Jabboury K et al: Cis-diamminedichloroplatinum(II) by 5-day continuous infusion: a new dose schedule with minimal toxicity. Cancer 53: 837-840, 1984

18.   Posner MR, Skarin AT, Clark J et al: Phase I study of continuous-infusion cisplatin. Cancer Treat Rep 70: 847-850, 1986

19.   Belliveau JF, Posner MR, Ferrari L et al: Cisplatin administered as a continuous 5-day infusion: plasma platinum levels and urine platinum excretion. Cancer Treat Rep 70: 1215-1217, 1986

20.   Posner MR, Ferrari L, Belliveau JF et al: A phase I trial of continuous infusion cisplatin. Cancer 59: 15-18, 1987

21   Forastiere AA, Belliveau JF, Goren M P et al: Pharmacokinetic and toxicity evaluation of five-day continuous infusion versus intermittent bolus cis-diamminedichloroplatinum(II) in head and neck cancer patients. Cancer Res 48: 3869-3874, 1988

22.   Nieweg R, Greidanus J, de Vries EGE: A patient education program for a continuous infusion regimen on an outpatient basis. Cancer Nursing 10: 177-182, 1987

23.   Mulder POM, de Vries EGE, Uges DRA et al: Pharmacokinetics of carboplatin at a dose of 750 mg/m^2 divided over three consecutive days. Br J Cancer (in press)

24.   Greidanus J, de Vries EGE, Nieweg MB et al: Evaluation of a totally implanted venous access port and portable pump in a continuous chemotherapy infusion schedule on an outpatient base. Eur J Cancer Clin Oncol 23: 1653-1657, 1987

25.   Foster BJ, Clagett-Carr K, Leyland-Jones B et al: Results of NCI-sponsored phase I trials with carboplatin. Cancer Treat Reviews 12: 43-49, 1985 (Suppl A)

26.   WHO Handbook for reporting results of cancer treatment. WHO Offset Publication no.48, Nijhoff Den Haag, 1978.

27.   Donker AJM, van der Hem GK, Sluiter WJ et al: A radioisotope method for simultaneous determination of the glomerular filtration rate and the effective renal plasma flow. Neth J Med 20: 97-103, 1977

28.   Speth PAJ, Linssen PCM, Boezeman JBM et al: Quantification of anthracyclines in human hematopoietic cell subpopulations by flow cytometry correlated with high pressure liquid chromatography. Cytometry 6: 143-150, 1985

29.   Speth PAJ, Linssen PCM, Beex LVAM et al: Cellular and plasma pharmacokinetics of weekly 20 mg 4'-epi-adriamycin bolus injection in patients with advanced breast carcinoma. Cancer Chemother Pharmacol 18: 78-82, 1986

30.   Leroy AF, Wehling ML, Sponseller HL et al: Analysis of platinum in biological materials by flameless atomic absorption spectrometry. Biochem Med 18: 184-191, 1977

31.   Terheggen PMAB, Dijkman R, Begg AC et al: Monitoring of interaction products of cis-diamminedichloroplatinum(II) and cis-diammine(1, 1-cyclobutanedicarboxylato)platinum-(II) with DNA in cells from platinum-treated cancer patients. Cancer Res. 48: 5597-5603, 1988

32. Scaf AHJ: Pharmacokinetic analysis with rugfit; an interactive pharmacokinetic computer program. Biopharm Drug Disp 9: 415-416, 1988

33. Loo JCK, Riegelman S: Assessment of pharmacokinetic constants from postinfusion blood curves obtained after iv infusion. J Pharm Sci 59: 53-55, 1970

34. Boxenbaum HG, Riegelman S, Elashoff RM: Statistical estimations in pharmacokinetics. J Pharmacokin Biopharm 2: 123-148, 1974

35. Leyvraz S, Ohnuma T, Lassus M et al: Phase I study of carboplatin in patients with advanced cancer, intermittent intravenous bolus, and 24-hour infusion. J Clin Oncol 3: 1385-1392, 1985

36. Rozencweig M, Nicaise C, Beer M et al: Phase I study of carboplatin given on a five-day intravenous schedule J Clin Oncol 1: 621-626, 1983

37. Van Echo DA, Egorin MJ, Whitacre MY et al: Phase I clinical and pharmacologic trial of carboplatin daily for 5 days. Cancer Treat Rep 68: 1103-1114, 1984

38. Bacha DM, Caparros-Sison B, Allen JA et al: Phase I study of carboplatin (CBDCA) in children with cancer. Cancer Treat Rep 70: 685-689, 1986

39. Shea TC, Flaherty M, Elias A et al: A phase I clinical and pharmacokinetic study of carboplatin and autologous bone marrow support. J Clin Oncol 7: 651-661, 1989

40. Koeller JM, Trump DL, Tutsch KD et al: Phase I clinical trial and pharmacokinetics of carboplatin (NSC-241240) by single monthly 30-minute infusion. Cancer 57: 222-225, 1986

41. Colombo N, Speyer JL, Green M et al: Phase II study of carboplatin in recurrent ovarian cancer: severe hematologic toxicity in previously treated patients. Cancer Chemother Pharmacol 23: 323-328, 1989

42. Joss RA, Kaplan S, Goldhirsch A et al: A phase I trial of cis-diammine-1, 1-cyclobutane dicarboxylate platinum II (carboplatin, CBDCA, JM-8) with a single dose every five week schedule. Invest New Drugs 2: 297-304, 1984

43. Cantwell BMJ: A phase II study of the platinum analogues JM8 and JM9 in malignant pleural mesothelioma. Cancer Chemother Pharmacol 18: 286-288, 1986

44. de Andres Basauri L, Pousa AL, Alba E et al: Carboplatin, an active drug in advanced head and neck cancer. Cancer Treat Rep 70: 1173-1176, 1986

45. Eisenhauer EA, Swenerton KD, Sturgeon JFG et al: Phase II study of carboplatin in patients with ovarian carcinoma: a national cancer institue of Canada clinical trials group study. Cancer Treat Rep 70: 1195-1198, 1986

46. Beer M, Cavalli F, Kaye SB et al: A phase II study of carboplatin in advanced or metastatic stomach cancer. Eur J Cancer Clinical Oncol 10: 1565-1567, 1987

47. Long HJ, Pfeifle DM, Wieand HS et al: Phase II evaluation of carboplatin in advanced endometrial carcinoma. J Natl Cancer Inst 80: 276-278, 1988

48. Akaza H, Hagiwara M, Deguchi N et al: Phase II trial of carboplatin in patients with advanced germ-cell testicular tumors and transitional cell carcinomas of the urinary tract. Cancer Chemother Pharmacol 23: 181-185, 1989

49. Van Glabbeke M, Renard J, Pinedo HM et al: Iproplatin and carboplatin induced toxicities: overview of phase II clinical trials conducted by the EORTC early clinical trials cooperative group (ECTG). Eur J Cancer Clin Oncol 24: 255-262, 1988

50. Sleijfer DTh, Smit EF, Meijer S et al: Acute and cumulative effects of carboplatin on renal function. Br J Cancer 60: 56-6, 1989

51. Meijer S, Sleijfer DTh, Mulder NH et al: Some effects of combination chemotherapy with cis-platinum on renal function in patients with nonseminomatous testicular carcinoma. Cancer 51: 2035-2040, 1983

52. Offerman JJG, Meyer S, Sleijfer DTh et al: Acute effects of cisdiammine-dichloroplatinum (CDDP) on renal function. Cancer Chemother Pharmacol 12: 36-38, 1984

53. Hardy JR, Tan S, Fryatt I et al: How nephrotoxic is carboplatin? Br J Cancer 61: 644, 1990
54. Egorin MJ, Van Echo DA, Tipping SJ et al: Pharmacokinetics and dosage reduction of cis-diammine(1, 1-cyclobutanedicarboxylato)platinum in patients with impaired renal function. Cancer Res 44: 5432-5438, 1984
55. Calvert AH, Newell DR, Gumbrell LA et al: Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 11: 1748-1756, 1989
56. Calvert AH, Harland SJ, Newell DR et al: Early clinical studies with cis-diammine-1, 1-cyclobutane dicarboxylate platinum II Cancer Chemother Pharmacol 9: 140-147, 1982
57. Harland SJ, Newell DR, Siddik ZH et al: Pharmacokinetics of cis-diammine-1, 1-cyclobutane dicarboylate platinum(II) in patients with normal and impaired renal function. Cancer Res 44: 1693-1697, 1984
58. Reece PA, Bishop JF, Olver IN et al: Pharmacokinetics of unchanged carboplatin (CBDCA) in patients with small cell lung carcinoma. Cancer Chemother Pharmacol 19: 326-330, 1987
59. Van Hennik MB, van der Vygh WJF, Klein et al: Comparative pharmacokinetics of cisplatin and three analogues in mice and humans. Cancer Res 47: 6297-6301, 1987
60. Lee EJ, Egorin MJ, Van Echo DA et al: Phase I and pharmacokinetic trial of carboplatin in refractory adult leukemia. J Natl Cancer Inst 80: 131-135, 1988
61. Gaver RC, Colombo N, Green MD et al: The disposition of carboplatin in ovarian cancer patients. Cancer Chemother Pharmacol 22: 263-279, 1988
62. Van Echo DA, Egorin MJ, Aisner J: The pharmacology of carboplatin. Sem Oncol 16: 1-6, 1989 (Suppl 5)
63. Calvert AH, Harland SJ, Newell DR et al: Phase I studies with carboplatin at the Royal Marsden Hospital. Cancer Treat Reviews 12: 51-57, 1985 (Suppl A)
64. Greidanus J, de Vries EGE, Mulder NH et al: A phase I and pharmacokinetic study with 21 days continuous infusion with mitoxantrone. J Clin Oncol 7: 797-797, 1989
65. Behrens RC, Hamilton TC, Masuda H et al: Characterization of a cis-diamminedichloro-platinum(II)-resistant human ovarian cancer cell line and its use in evaluation in platinum analogues. Cancer Res 47: 414-418, 1987
66. Hospers GAP, Mulder NH, de Jong B et al: Characterization of a human small cell lung carcinoma cell line with acquired cisplatin resistance in vitro. Cancer Res 48: 6803-6807, 1988
67. Reed E, Ozols RF, Tarone R et al: Platinum-DNA adducts in leukocyte DNA correlate with disease response in ovarian cancer patients receiving platinum-based chemotherapy. Proc Natl Acad Sci USA 84: 5024-5028, 1987.
68. Reed E, Yuspa SH, Zwelling LA et al: Quantitation of cis-diamminedi-chloroplatinum II (cis-platin)-DNA-interstrand adducts in testicular and ovarian cancer patients receiving cisplatin chemotherapy. J Clin Invest 77: 545-550, 1986
69. Fichtinger-Schepman AMJ, van Oosterom AT, Lohman PHM et al: Cis-diamminedichloro-platinum(II)-induced DNA adducts in peripheral leukocytes from seven cancer patients: quantitation immunochemical detection of the adduct induction and removal after a single dose of cis-diammine-dichloroplatinum(II). Cancer Res 47: 3000-3004, 1987.

PHASE I STUDY OF 21 DAYS CONTINUOUS INFUSION WITH VINDESINE

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Vindesine (VDS) is a semi-synthetic vinca alkaloid. Continuous infusion schedules have resulted in an improved therapeutic index for this, as well as for related alkaloids (16, 10, 8). Continuous administration of the drug over a few days allowed augmentation of the dosage of VDS without increasing toxicity. Responses were seen with continuous infusion in tumors resistant to bolus injections of vinca alkaloids (11, 12). The rationale for such schedules has been based on the relatively short plasma half life of VDS in pharmacological studies (9), and the fact that vinca alkaloids are cell cycle specific drugs. We performed a study of a 21 day continuous infusion of VDS administered with an ambulatory pump.

Eligibility criteria for the study were: age 21-75 years, no prior treatment with vinca alkaloids, normal blood count, bilirubin < 35 mmol/l, creatinine < 130 μmol/l, absence of neurological disorder and Karnofsky score > 60. For continuous infusion an implantable venous access port (7) (Infuse A Port) and a portable pump (Graseby Medical MS 16A^{tm} syringe driver) were used. A 20 ml luer lock syringe with VDS dissolved in 0.9% NaCl was connected to the port via an extension tube and a Huber point needle (7). Patients formulated the VDS at home and replaced syringes every other day in order to assure drug stability. Treatment was performed on an outpatient basis as described before (5). A starting dose of 0.2 mg/m²/day for 21 days VDS was chosen, followed by a 3 weeks rest period. Toxicity was evaluated according to WHO, on days 7, 14, 21, 31 and 42 since start of therapy. Unacceptable toxicity and therefore abolishment of treatment was defined as neurotoxicity grade 2 and/or any other parameter reaching grade 3 toxicity according to the WHO grading system (15). For pharmacokinetic analysis blood samples were drawn at 19 hrs, 40 hrs, 8, 13 and 14 days after start and 24 hrs after cessation of therapy. The concentration of VDS in plasma was determined with HPLC and electrochemical detection according to Vendrig et al. (14).

In four patients (see table I for patient characteristics) therapy with VDS, dose 0.2 mg/m²/day dose was started. Hematological toxicity was limited, only one patient developed leukopenia grade 1. Liver function disturbances due to VDS were not seen.

Neurotoxicity was the major complication and led to drug withdrawal in 3 patients during the first course, whereas the fourth patient did not have any sign of neurotoxicity. In the second week of treatment these 3 patients started to complain about pain in the legs and knees and progressive muscle weakness. They had problems climbing staircases or walking more than a few meters, despite cessation of treatment. Eventually one patient was so severely disabled that she had to use a wheel-chair. After cessation of therapy muscle strength was regained in all patients over a period of 3-4 weeks. Two of these three patients had in addition severe one-sided jaw pain, and one had low back pain and the feeling as if being battered. Jaw pain started also in the second week, the patient needed analgesics, and the pain subsided after treatment discontinuation. None complained about paresthesias in the upper or lower extremities or had so on physical examination. Signs of autonomic neuropathy were not seen.

Plasma concentrations of VDS were determined in patient number 1 (table I). After 19 and 40 hrs and 8 days infusion the concentrations of the samples were 0.5, 0.6 and 0.8 ng VDS/ml, respectively. The concentration of VDS in samples taken during infusion (days 13, 14) and after the cessation of therapy were below the determination limit.

Table I.   Patient characteristics.

Number	Sex	Age	Diagnosis	Days of treatment	Total cumulative dose administered
1	F	68	Renal cell carcinoma	14	4.3 mg
2	F	61	Renal cell carcinoma	16	5.6 mg
3	M	56	Gastric carcinoma	21	9.45 mg
4	F	42	Malignant melanoma	19	7.0 mg

In various studies with VDS (16, 12, 11, 2, 4) it is shown that 5 day continuous infusion makes it possible to administer a higher drug dose with similar or less toxicity compared to bolus injection. Dose limiting side effects were hematologic and neurological toxicity. Been et al. (1) found more profound neurotoxicity with repeated courses. Gralla et al. (6) found, in a weekly VDS schedule, that the degree of neuropathy was related to the total dose of VDS received. In one of these studies muscle pain, arthralgias, jaw pain and the feeling of being battered were described as side effect.

In a study with prolonged infusion of vinblastine (median duration 30 days, maximum 81 days) no neurological toxicity was seen (10). Dose limiting side effect was leukopenia and maximum tolerated dose 0.75 mg/m^2/day vinblastine for 30 days. In our study, the intended 0.2 mg/m^2/day for 21 days could not be administered in three out of four patients due to neurotoxicity. The total dose administered in these patients (mean 5.6 mg) if administered as bolus injection does usually not lead to neurotoxicity. Pharmacokinetic analysis performed in one patient, showed no plasma accumulation of VDS during the 14 days treatment period of the patient. This does, however, not exclude accumulation of the drug in nerve of muscle tissue. In a pharmacokinetic analysis of a 5 days infusion regimen (9)

there was also no accumulation of the drug found. The area under the curve (AUC) calculated for a patient with a hypothetical steady state plasma level of 0.8 ng/ml (the highest level detected in one of our patients) is still smaller than the AUC in the 2 day infusion (total dose 5.4 mg) or bolus injection (total dose 4.0-5.0 mg) found by others (9, 13). In the study of Jackson (11) only mild neurotoxicity was seen.

It can be concluded that VDS cannot be administered safely for 21 days at the low dose of 0.2 mg/m^2/day. Therefore VDS infusion for longer periods does not seem to result in a more favorable dose/toxicity ratio. Due to severe neurotoxicity this regimen is not recommended for further studies.

REFERENCES

1. Been, M., Vanini, M., Cavelli, F. (1980). A study of the toxicity of vindesine in continuous infusion over five days. In: Proceedings of the International Vinca Alkaloid Symposium - Vindesine, Brade, W., Nagel, G.A., Seeber, S. (eds) p. 78. S.Karger, Basel.
2. Bodey, G.P., Yap, H.Y., Yap, B.S., Valdivieso, M. (1980). Continuous infusion vindesine in solid tumors. Cancer Treat.Rev., 7 (suppl.), 34.
3. Bodey, G.P., Yap, H.Y., Blumenstein, G.R., Savaraj, H., Loo, T.L. (1980). Continuous infusion of vindesine in breast carcinoma clinical and pharmacological studies. In Proceedings of the International Vinca Alkaloid Symposium - Vindesine, Brade, W., Nagel, G.A., & Seeber, S. (eds) p. 203. S.Karger, Basel.
4. Carlson, R.W., Sikic, B.I. (1983). Continuous infusion or bolus injection in cancer chemotherapy. Ann.Int.Med., 99, 823.
5. De Vries, E.G.E., Greidanus, J., Mulder, N.H., (1987). A phase I and pharmacokinetic study with 21 day continuous infusion of epirubicin. J.Clin.Oncol., 5, 1445.
6. Gralla, R.J., Casper, E.S., Kelsen, D.P., (1981). Cisplatin and vindesine combination chemotherapy for advanced carcinoma of the lung: a randomized trial investigating two dosage schedules. Ann.Int.Med., 95, 414.
7. Greidanus, J., de Vries, E.G.E., Nieweg, M.B., de Langen, Z.J., Willemse, P.H.B. (1987). Evaluation of a totally implantable venous access port and portable pump in a continuous chemotherapy infusion schedule on an outpatient basis. Eur.J.Cancer Clin.Oncol., 23, 1653.
8. Jackson, D., Paschold, E.H., Spurr, C.L., (1984) Treatment of advanced non Hodgkin's lymphoma with vincristine infusion. Cancer, 53, 2601.
9. Jackson, D.V., Sethi, V.S., Long, T.R., Muss, H.B., Spurr, C.L. (1984). Pharmacokinetics of vindesine bolus and infusion. Cancer Chemother. Pharmacol., 13, 114.
10. Lokich, J.J., Zipoli, T.E., Perri, J., Bothe, A. (1984). Protracted vinblastine infusion. Phase I-II study in malignant melanoma and other tumors. Am.J.Clin.Oncol. (CCT), 7, 551.
11. Mascret, B., Maranchini, D., Tubiana, N., Gastaut, J.A., Carcassone, Y. (1983). Continuous 5-day infusion of vindesine in patients with refractory malignant lymphomas. (Abstr) 2nd European Conference on Clinical Oncology and Cancer Nursing, 07-22.
12. Mathé, G., Misset, J.L., De Vassal, F. (1978). Phase II clinical trial with vindesine for remission induction in acute leukemia, blastic crises of chronic myeloid leukemia, lymphosarcoma and Hodgkin's disease: Absence of cross resistance with vincristine. Cancer Treat.Rep., 62, 805.
13. Rahmani, R., Kleisbauer, J.P., Cano, J.P., Martin, M., Baubet, J. (1985). Clinical pharmacokinetics of vindesine infusion. Cancer Treat.Rep., 69, 839.

14. Vendrig, D.E.M.M., Teeuwsen, J., Holthuis, J.J.M. (1988). Analysis of vinca alkaloids in plasma and urine using high-performance liquid chromato-graphy with electrochemical detection. J.Chromatogr., 424, 83.
15. WHO Handbook for reporting results of cancer treatment (1978). WHO Offset Publication no.48, Nijhoff, Den Haag.
16. Yap, H.Y., Blumenschein, G.R., Bodey, G.P., Hortobagyi, G.H., Buzcker, A.U., Distefano, A. (1981). Vindesine in the treatment of refractory breast cancer: Improvement in therapeutic index with continuous 5-day infusion. Cancer Treat.Rep., 65, 775.

IN VITRO RESPONSE OF HUMAN SMALL CELL LUNG CANCER CELL LINES TO CHEMOTHERAPEUTIC DRUGS; NO CORRELATION WITH CLINICAL DATA[a]

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SUMMARY

Three cell lines derived from tumors of patients who had no clinical response after treatment with a multi drug regimen were compared to three cell lines derived from tumors of patients who, upon treatment, showed a clinically complete response. These two groups of cell lines were considered to represent the in vitro counterparts of the two extremes of the clinical spectrum of sensitivity for chemotherapeutic drugs in small cell lung cancer. To assess whether the in vivo (in)sensitivity of a tumor to a certain drug regimen is retained in vitro, the cell lines were tested for drug sensitivity using the micro titerwell tetrazolium assay and the results were compared with the in vivo data. No correlation was found. Since in vitro models using cell lines are based on the assumption that a cell line reflects the properties of the tumor from which it is derived several additional parameters such as monoclonal antibody staining pattern and DNA content were analyzed in the biopsies and the cell lines. The results show that selection of discrete cell populations during in vitro culture may occur.

Abbreviations

SCLC	=	small cell lung cancer.
CR	=	complete response.
FCS	=	fetal calf serum.
MTA	=	MTT assay
MTT	=	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide.
PBS	=	phosphate buffered saline (0.14 M MaCl, 2.7 mM KCl, 6.4 mM $Na_2HPO_4.2H_2O$ and 1.5 mM KH_2PO_4, pH 7.4).
MoAb	=	monoclonal antibody.
MDR	=	multi drug resistance.
TBS	=	tumor biopsy specimen.

INTRODUCTION

Small cell lung cancer (SCLC) accounts for 20-25% of newly diagnosed cases of lung cancer (1). It is characterized by the early and widespread occurrence of metastases and rapid progression of disease. Human SCLC provides a good model to study the problems of initially unresponsive tumors. The vast majority, about 90-95%, of patients with SCLC show major responses to chemotherapeutic agents of different classes (2). However, 5-10% of patients present with a tumor that is initially unresponsive to most commonly used cytotoxic agents. Invariably, these patients die within several weeks. Tumors of the latter group of patients may be designated intrinsically resistant in terms of the Goldie-Goldman hypothesis, as opposed to the initially sensitive tumors that are present in the former group (3). The aim of the present investigation is to study if this *in vivo* phenomenon is also reflected *in vitro*.

MATERIALS AND METHODS

Chemicals
Doxorubicin was provided by Farmitalia Carlo Erba, Milan, Italy. Cisplatin and Etoposide were obtained from Bristol Myers S.A.E., Madrid, Spain, Vincristine (Oncovin) from Eli Lilly S.A., St. Cloud, France and Melphalan from Wellcome, London, Great Britain. Fetal calf serum was from Flow Laboratories, Irvine, Great Britain, RPMI was obtained from Gibco, Paisly, Great Britain. MTT was obtained from Sigma, St. Louis, MO.

Cell lines
Three cell lines GLC 3, GLC 12 and GLC 20 were established from patients who had no measurable response on chemotherapy. GLC 3 was derived from a pleural effusion of a 61 year old male and has been described before (4). In short the clinical course of this patient was as follows: he received two courses of Cyclophosphamide (750 mg/m^2) day 1, Cisplatin (75 mg/m^2) day 1 and Etoposide (100 mg/m^2) day 1,3,5 q 3 weeks. Since no response was seen, chemotherapy was changed then to Doxorubicin (60 mg/m^2) day 1, Vincristine (1 mg/m^2) day 1 and 8 and Procarbazine (100 mg/m^2) days 1-10 q 3 weeks. After two courses of second line chemotherapy the tumor was still clearly progressive and at that time GLC 3 was established from the pleural effusion. The patient died 14 weeks after the start of chemotherapy. GLC 12 was derived from a 64 year old male who received four courses of Cyclophosphamide (1000 mg/m^2) day 1, Doxorubicin (40 mg/m^2) day 1 and Vincristine (1 mg/m^2) days 1 and 8 q 3 weeks. The tumor showed no change on chest X-rays. A thoracotomy was performed and a lymph node metastases was found adherent to the pulmonary artery. From this lymph node GLC 12 has been established. Radiotherapy (37.5 Gy) was administered to the left hilar region and mediastinum, which again induced no measurable tumor regression. This patient died with extensive liver metastases 33 weeks after start of chemotherapy. GLC 20 was derived after 6 courses of

chemotherapy consisting of Cyclophosphamide (1000 mg/m^2) day 1, Doxorubicin (40 mg/m^2) day 1 and Vincristine (1 mg/m^2) days 1 and 8 q 3 weeks. The tumor of this 67 year old male showed no regression and became progressive within a few weeks after chemotherapy had been stopped. A biopsy was taken of the primary tumor by broncho-scopy and the cell line made. The patient died 28 weeks after the diagnosis of SCLC. The cell lines GLC 14, GLC 22 and GLC 33 were established from patients who showed cli-nically complete remissions (CR) after chemotherapy. The patient history of GLC 14 has been described elsewhere (5,6). In short, GLC 14 was established from a supraclavicular lymph node of a 55 year old female which was removed before chemotherapy. After five courses of Cyclophosphamide (1000 mg/m^2) day 1, Doxorubicin (45 mg/m^2) day 1 and Etoposide (100 mg/m^2) days 1,3,5 q 3 weeks a clinically CR was reached. The tumor relapsed after a 31 weeks disease free interval and the patient died 86 weeks after start of chemotherapy. GLC 22 was derived from the primary tumor site (right lower lobe) of a 50 year old male prior to chemotherapy. The same chemotherapy regimen as indicated for the patient from which GLC 14 was established was administered. A clinical CR was achieved after five courses. This patient died from brain metastases 71 weeks after the diagnosis of SCLC. GLC 33 was established from a 62 year old female with a supracla-vicular lymph node metastases present at the time of diagnosis. After two courses of che-motherapy as described under GLC 14, a CR was reached, the patient received addition-ally 3 courses. Eventually she died 47 weeks after the start of chemotherapy.

The cell lines were maintained in of 5% CO2, 95% air at 37 ^{0}C. GLC 3, GLC 20 and GLC 14 were kept in culture in RPMI 1640 medium supplemented with 10% heat inacti-vated fetal calf serum (FCS). The growth medium for GLC 12, GLC 22 and GLC 33 was SCLC$_2$ medium made according to the method of Minna et al (7). In addition the medium was supplemented with 3% FCS. GLC 3, GLC 14 and GLC 33 grow partly attached and partly floating in aggregates. GLC 12, GLC 20 and GLC 22 grow exclusively floating in aggregrates. The culture doubling time for GLC 3, GLC 12 and GLC 20 was 25, 72 and 49 hrs and for GLC 14, GLC 22 and GLC 33 26, 44 and 32 hrs. For all experiments, cells were incubated 1:1 for 5 min with 0.02% EDTA solution (0.35 g NaHCO3, 8 g NaCl, 0.4 g KCl, 1 g dextrose, 0.2 g EDTA, 1.0 l H$_2$O) to prepare a cell suspension containing small clumps with a viability as determined by trypan blue exclusion of >75% for all cell lines. All cell lines were tested between passages 20 and 50.

MTT assay (MTA)

The MTA as described by Mossman (8) and Carmichael et al (9) was used with some minor modifications (10). Before the assays were performed a linear relationship of cell number to MTT formazan crystal formation was checked. The relationship between see-ding density, incubation volume and incubation period was established for each cell line separately after growth studies. Optimal seeding densities were 10.000 cells/well (GLC 20), 20.000 cells/well (GLC 33), 25.000 cells/well (GLC 12, GLC 22), 30.000 cells/well (GLC 3) and 35.000 cells/well (GLC 14). Total volume of incubation was 100 µl (GLC 3, 14 and 20) and 250 µl (GLC 12, 22 and 33). Culture periods were 4 days (GLC 14), 5

days (GLC 3), 8 days (GLC 33), 11 days (GLC 20), 16 days (GLC 12) and 22 days (GLC 22). Plates containing GLC 12 and 22 were centrifuged at 180 g for 10 min on day 7 (GlC 12 and GLC 22) and day 14 (GLC 22), 100 µl of medium were removed from each well, after which 100 µl of fresh medium were added again. In this way, it was assured that all cell lines had been cultured in the test plates for two to three cell divisions and were in exponentional growth phase at the time of testing. The appropriate number of cells was incubated in 96-well cultureplates (nunc. Gibco) in culture medium to which one of the following agents was added for 1 hr: Doxorubicin, Cisplatin, Vincristine, Etoposide, Melphalan. All drug stock solutions were diluted with RPMI 1640 medium to reach the desired concentrations. Melphalan was used as a substitute for Cyclophosphamide as Melphalan does not require endogenous activation (11). For X-Ray treatment (Philips X-Ray source operated at 200 kV, 15 mA, 0.5 mm Cu/A filter) cells were placed in plastic tubes in a volume of 500 µl. After irradiation the cells were transferred to 96-well culture plates. Combination chemotherapy was simulated *in vitro* by incubating each cell line with a combination of drugs. To this aim the separate drug concentration resulting in 10% growth inhibition (ID_{10}) for each cell line was determined. Then the cell lines were incubated with combination of drugs, using drug concentrations at the prior established ID_{10} for each individual drug.

After incubation, cells were washed three times with fresh medium by centrifugation of the microtiter plates at 180 g for 10 min. Following the subsequent culturing period (for different time periods for different cell lines as determined before) 20 µl of MTT solution (5 mg/ml PBS) were added to each well and the cells were incubated at 37 °C for 165 min. Thereafter the plates were centrifuged at 180 g for 15 min and the supernatant was carefully removed without disturbing the formazan crystals. Cells grown in the $SCLC_2$ medium (GLC 12, GLC 22, GLC 33) were washed once with PBS. This step was introduced because this medium caused a turbidity with dimethyl sulfoxide probably due to bovine serum albumin. Subsequently the formazan crystals were solubilized by adding 200 µl 100% dimethyl sulfoxide. All plates were scanned at 520 nm immediately after resolubilizing of the formazan crystals using a scanning microtiterwell spectrophotometer (Titertek Multiscan, Flow lab). The percentage cell survival was calculated by the formule: mean of the test samples/ mean of three untreated samples. Controls consisted of media without cells (background extinction) and cells incubated in wells with medium only. For each drug, drug combination and X-Ray treatment at least three experiments were performed, each in quadruplicate.

Cellular Doxorubicin level

Cellular Doxorubicin levels were determined after 1 hr incubation in appropriate medium at 37°C, using at least 1×10^6 cells. To correct for extracellular bound Doxorubicin control samples were incubated at 0°C for 5 min. The incubation period was terminated by washing the cells three times with ice cold PBS, and extracted with 0.3 N HCl-50% overnight. After centrifugation the fluorescence was measured in the supernatant in a Kontron spectrofluorometer at excitation and emission wavelenghts of 474 and 549 nm respectively

(12). Intracellular Doxorubicin levels were calculated by substracting the values measured at 0°C from the corresponding levels measured at 37°C.

Tumor biopsy specimen
The original tumor biopsy specimen from which the cell lines were derived, except GLC 3, which was derived from a pleural effusion, were split in three. One part was used for routine pathological procedures, one part for establishment of the cell line and a third part was snap frozen in OCT compound in liquid freon and kept at -80°C until use. This last part was used for immunohistological examination of the tumor cells with monoclonal antibodies and DNA flow cytometry. Normal histological examination showed that the tumor biopsy specimen associated with GLC 12 and GLC 14 consisted of almost pure tumor material, the remaining tumor biopsy specimen were admixed with normal tissue, but consisted for at least of 25% tumor cells. All tumor biopsy specimen were immunostained with monoclonal antibodies, whereas DNA flow cytometric analysis was possible only with two specimen, namely tumor biopsy specimen of GLC 12 and GLC 22.

Monoclonal antibodies (MoAb)
Cryostat sections from frozen tumor biopsy specimen were made. Immunostaining was performed on these sections and on cytospin preparations of cell lines according to previously described methods (13). MoAb directed against different SCLC associated antigens have been generated by one of us (L.de L.). These antibodies are reactive with antigens expressed in normal and malignant tissues with a neuroendocrine differentiation state (MOC-1, MOC-21, MOC-32, MOC-51, MOC-52) (14-16) and an antibody reactive with tissues with an epithelial differentiation state (MOC-31). Three MoAb directed against intermediate sized filament proteins were also included in the study. RGE 53, directed against cytokeratin 18 (17), Vim, directed against vimentin (18) and MNF, a MoAb directed against the 210 and 68 kD components of neurofilaments (19,20). Vim and MNF are non-reactive with normal bronchial epithelia. RGE 53 reacts with normal bronchial columnar epithelium and type 2 pneumocytes. Also leu-7, a MoAb directed against an natural killer cell associated antigen (21) was tested. The expression of the 170 kD P-Glycoprotein was investigated using two antibodies; C-219 and JSB-1 as developed by Ling et al.(22) and Scheper et al.(23) respectively.

DNA Flow Cytometry
Frozen biopsy specimen were processed using the detergent-trypsin method developed by Vindelov et al (24,25). From the specimen from which GLC 12 and GLC 22 were established and the six cell lines, nuclei were isolated and stained with propidium iodide. Flow cytometry was performed with an Ortho ICP flow cytometer. Trout red blood cells were stained with propidium iodide and used as an internal reference for all samples. A cryostat section was made from the same biopsy to check cell composition of the samples histologically.

RESULTS

In vitro drug sensitivity

Cell survival curves are shown in figures 1-6. In table I the drug concentration and X-ray treatment resulting in 50% growth inhibition (ID_{50}) relative to GLC 12, for practical purposes, is calculated. Also depicted are the results of combination of drugs relative to GLC 12 at prior established ID_{10}. For all drugs tested there was a wide variation in sensitvity, e.g. for

Table I. X-ray (rad) and drug concentration resulting in 50% growth inhibition and cell survival following combination of drugs at prior established ID_{10} (see text). Numbers are relative to GLC 12.

GLC	12*	3*	20*	14**	22**	33**
X-ray	315 rad	1.4	1.5	1.1	0.7	1.1
Adriamycin	0.1 nM	180	190	650	33	35
Cisplatin	650 nM	369.2	115.4	1538.5	12.3	17.2
VP 16-213	380 nM	8.3	3.2	>138.9	0.6	2.1
Vincristine	946 nM	33.3	3.2	67.0	1.0	1.4
Melphalan	131 nM	32.1	7.8	178.5	1.9	0.9
Combination[1]	74 %[2]	1.09	0.93	1.04	0.97	0.9

*   post-treatment derived from non-responding patients,
**   pre-treatment derived from patients showing complete response after chemotherapy.
[1]   Drugs used in combination were: Melphalan, Cisplatin and Vp 16-213 for GLC 3; Melphalan, Adriamycin and Vincristine for GLC 12 and GLC 20; Melphalan, Adriamycin and Vp 16-213 for GLC 14, GLC 22 and GLC 33.
[2]   Number is expressed as % of controls (see text).

Doxorubicin ID_{50} covered a 3 log range. Also, there was no correlation for sensitivity of individual drugs with the clinical data. GLC 12 proved to be the most sensitive for 3 out of 5 drugs tested, whereas it originated from a patient who had progressive disease despite chemotherapy. In contrast, GLC 14, derived from a patient who reached a clinical CR, was the most resistant for all drugs tested. Experiments using a combination of drugs also failed to show a dichotomy between cell lines derived from non responders versus complete responders. Drug testing at this level did not show differences between these two groups of cell lines.

Cellular Doxorubicin content

Cellular Doxorubicin content after 1 hr incubation with various concentrations is shown in figure 7. GLC 14 and GLC 3, cell lines relatively resistant to Doxorubicin compared to GLC 12 had the highest cellular Doxorubicin content. On the other hand, GLC 20 and GLC 3, cell lines with a comparable resistance factor relative to GLC 12, but GLC 20 had a significant lower cellular Doxorubicin content as GLC 3 at high incubation concentrations. Cellular Doxorubicin content after incubation concentrations close to the ID_{50} of the cell lines, i.e. 0.5 μM, did not reveal significant differences.
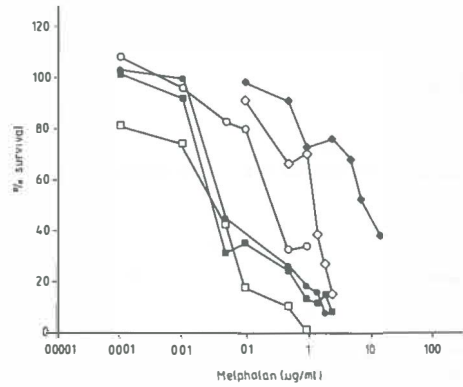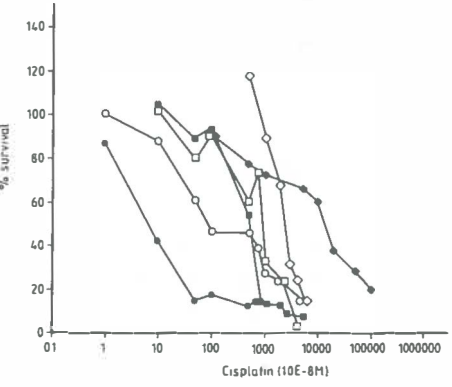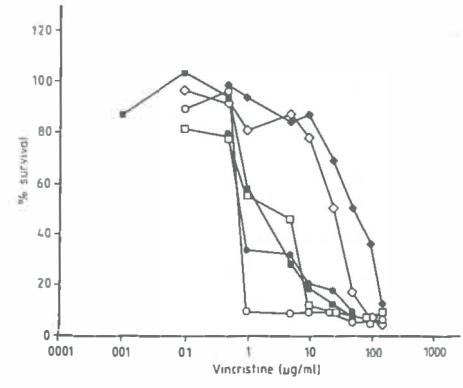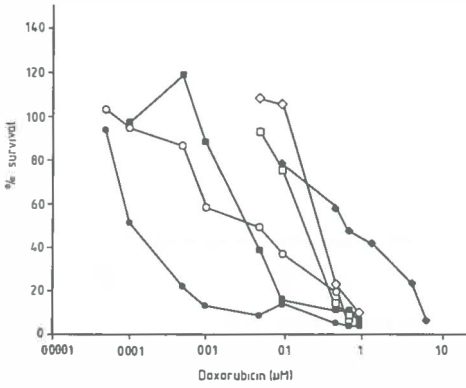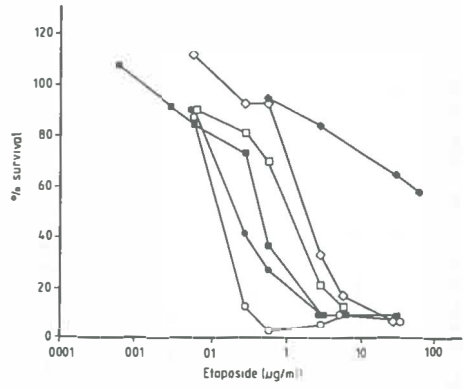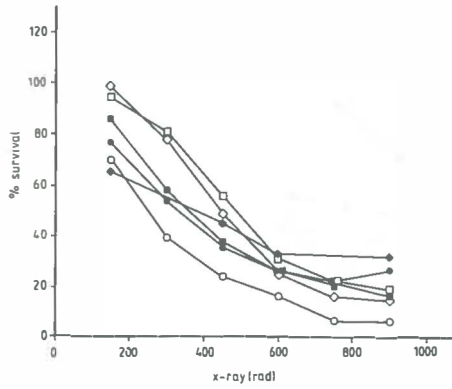
Figure 1-6. Micro titerwell tetrazolium assy after X-ray treatment (fig 1) and 1 hr incubation with Doxorubicin (fig 2), Cisplatin (fig 3), Etoposide (fig 4), Vincristine (fig 5) and Melphalan (fig 6).
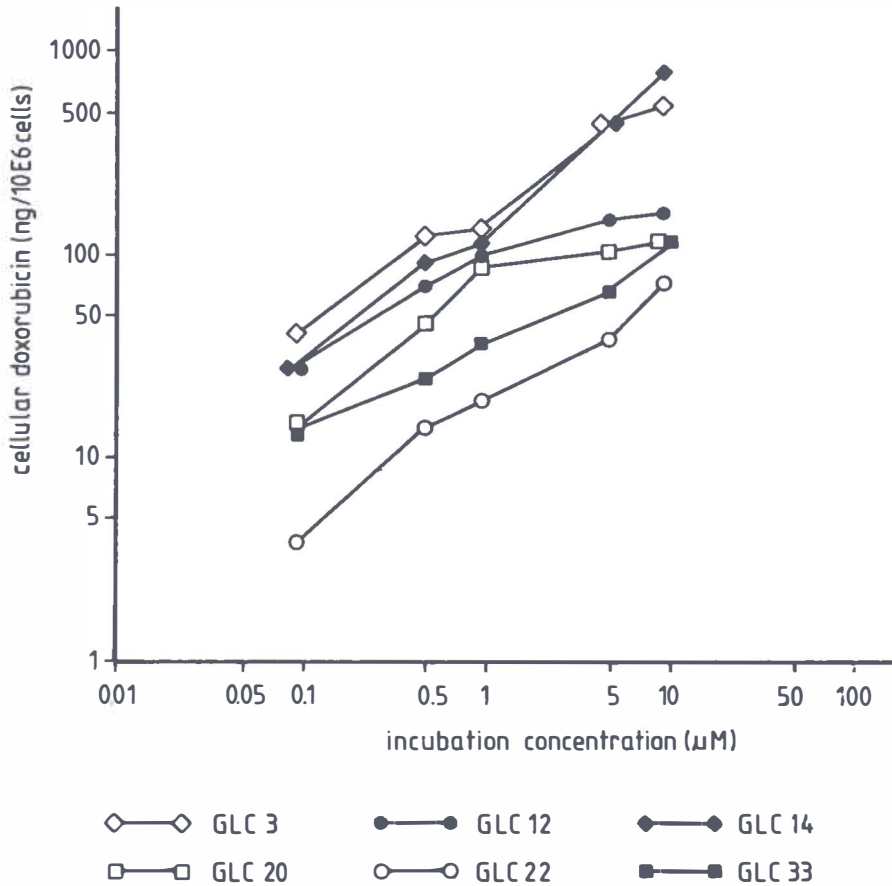
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Figure 7. Cellular Doxorubicin concentrations after 1 hr incubation with Doxorubicin.

Immunohistochemistry of tumor biopsy specimen and cell lines (Table II)

Original tumor biopsy specimen were available for all corresponding cell lines except for GLC 3. Immunohistochemical analysis revealed only minor differences between the tumor biopsy specimen and their corresponding cell lines. An antigen recognized by leu-7 was present on most tumor cells in culture, whereas it was invariably absent in the tumor biopsy specimen. Two MoAb reactive with intermediate filaments (MNF and Vim) showed positive staining with a small number of tumor cells in culture in all cell lines, with the exception of GLC 33 where there was no staining with Vim. In contrast, only in two tumor biopsy specimen, tumor biopsy specimen 20 and tumor biopsy specimen 14, tumor cells were present reactive with these two MoAb. The neuroendocrine related antigens recognized by MoAb MOC-1, MOC-21, MOC-32, MOC-51 and MOC-52 as well as the epithelial antigen detected by MOC-31 were present in tumor cells in culture and showed similar staining patterns in their related tumor biopsy specimen. Also, no evidence of expression of the multidrug resistance related 170 kD P-glycoprotein as detected by

the MoAb C-219 was found, neither in the tumor biopsy specimen nor in the cell lines. Staining of the cell lines and the tumor biopsy specimen with the MoAb JSB-1 showed positive intra-cytoplasmatic reactivity with GLC 3, GLC 20, GLC 14, GLC 22, and the tumor biopsy specimen 20 and 22. However, the number of cells reactive with JSB-1 varied with the passage of the cell line *in vitro* from total absence to 5-10% weak positive cells.

Table II. Immunostaining of cell lines and corresponding tumor biopsy specimen (TBS).

	GLC3	GLC12	TBS12	GLC20	TBS20	GLC14	TBS14	GLC22	TBS22	GLC33	TBS33
MNF	–	<1%	–	<10%	+	<1%	<10%	<1%	+	<1%	–
Vim	+f	<1%	–	<10%	–	<1%	<1%	<1%	–	–	–
RGE53	++	++	++	–	–	+	+	++	+	+	++
MOC1	++	++	++	+++	+++	++	++	++	++	++	+++
MOC21	++f	+	++	+	++	++	++	+	<10%	++	++
MOC31	++	+++	++	+++	+++	+++	++	+++	+f	+++	+++
MOC32	+	++	++	++	++	++	++	++	+	++	+++
MOC51	–	+	++	–	<10%	+f	+	+	+	++	++
MOC52	++f	+	++	+	+	+	++	+f	<10%	++	++
leu7	++	++	–	+++	–	++	–	+	–	+	<10%
C–219	–	–	–	–	–	–	–	–	–	–	–
JSB–1	+	–	–	+	<5%f	<5%f	–	<1%	<1%f	–	–

+	= <50%,
++	= 50-75%,
+++	= 75-100% of positive tumor cells,
f	= focal.

DNA flow cytometry

The results are shown in table III. In tumor biopsy specimen 12 two tumor cell populations as defined by their DNA index were present. These indexes were 1.19 and 1.53 respectively. In contrast, in GLC 12 only one tumor cell population with a DNA index of 1.55 could be detected. In GLC 22 and its related tumor biopsy specimen the reverse was

Table III. DNA index of cell lines and tumor biopsy specimen (TBS).

GLC 3	1.47		
GLC 12	1.55	TBS 12	1.19 - 1.53
GLC 20	2.07		
GLC 14	1.76		
GLC 22	1.05 - 1.95	TBS 22	1.75
GLC 33	1.00		

seen. In this tumor biopsy specimen one tumor cell population with DNA index of 1.75 was present. In GLC 22 two tumor cell populations with DNA indexes of 1.05 and 1.95 could be distinguished.

DISCUSSION

This study shows that results of drug sensitivity testing using the MTA performed on cell lines derived from patients with clinically resistant tumors and clinically sensitive tumors do not necessarily correlate with clinical data obtained from these patients in terms of tumor response to chemotherapeutic drugs, as can be inferred from figures 1-6.

There are only a few reports of comparisons between clinical response and *in vitro* tests using bronchial carcinomas. Berendsen et al.(5) described the *in vitro* response, using the fast green assay, of three cell lines, including GLC 14, obtained from one patient during longitudinal follow-up. In their material the pretreatment derived cell line, i.e. GLC 14, turned out to be the most sensitive cell line, whereas the cell line obtained immediately after induction chemotherapy was most refractory to some of the drugs tested. They concluded that the series of cell lines described might provide a relevant *in vitro* model to study (development of) drug resistance in the clinic. Retrospective studies using the MTA such as reported by Carmichael et al. (26) suggest that differences in tumor chemosensitivity between pretreated patients in contrast to untreated patients with SCLC may be simulated *in vitro*. Others, using smaller number of established cell lines found similar results (27,28).

Resistance to chemotherapeutic agents has been associated widely with the emergence of a 170 kD membrane glycoprotein, the so called P-glycoprotein (29). It acts as a drug efflux pump for several structurally unrelated drugs (30). Recent studies have shown that resistance mediated by P-glycoprotein is very unlikely to account for the majority of treatment failures in SCLC. Using slot blot analysis of MDR1 RNA encoding for P-glycoprotein, Goldstein and coworkers (31) were unable to detect MDR1 RNA in 21 SCLC cell lines. In experimental systems, like our own GLC/Adr SCLC cell line with a stable resistance factor of 44 for Doxorubicin, no evidence of expression of P-glycoprotein could be demonstrated (32). In the cell lines under discussion no evidence was found of the presence of P-glycoprotein as detected by C-219. Although in some cell lines and tumor biopsy specimen there was strong intracytoplasmatic reactivity with the MoAb JSB-1, the relevance of this finding is not clear (23). In the cell lines studied, those with the highest ID_{50} for Doxorubicin had the highest intracellular Doxorubicin content after one hour incubation (fig 7). Differences in cell size (33) and compartimentalization of Doxorubicin (34) may account for this phenomenom, but were not studied. An alternative explanation may be enhanced DNA repair capacity in the cell lines with relatively high Doxorubicin content and high ID_{50} for this drug in the MTA. This mechanism is known to be operative in several reported experimental systems (35,36).

The most likely explanation why the *in vivo* response differed from the *in vitro* response to cytotoxic drugs and X-ray treatment in our study is because apparently selection of tumor cell populations in culture has been taken place in at least 2/6 cell lines tested. Whether the tumor biopsy specimen are representative in this regard for the tumor as a whole in the patient is an additional question that cannot be answered. Nonetheless, we feel that a reasonable explanation for the observed differences of *in vitro* and *in vivo* sensitivity to cytotoxic drugs is due to selection of tumor cells in culture. Years ago, this phenomenon was already observed by Berry et al (37). Engelholm et al (38) report on the genetic instability of a single human SCLC cell line measured by serial DNA flow cytometric analysis. These results seems to be contradicted by two reports from the National Cancer Institute concerning small cell lung cancer cell lines (39,40). They concluded that tumor cells cultured *in vitro* retain their original DNA-indexes based on analysis of 65 cell lines. Notably they also stated that these cultured cells are thus useful for drug sensitivity analysis. However, some of the cell lines reported appear to have an unstable DNA-index after multiple passages *in vitro*. Therefore, although in general DNA contents of cell lines may reflect those of the tumors from which they were initiated, this may not hold true for all cell lines.

Immunohistochemical analysis of cell lines compared to original tumor biopsy specimen revealed only slight differences (Table I). In contrast to Berendsen et al.(41) who found loss of neuroendocrine differentiation antigens as detected by MOC-21 and MOC-32 in SCLC patients with relapsing disease and no response to chemotherapy, we found no meaningful differences between the 'sensitive' and 'resistant' cell lines and tumor biopsy specimen.

In conclusion, the results of our study show that chemosensitivity testing in cell lines using the MTA is not a proper tool to simulate clinical drug sensitivity *in vitro* for SCLC. This is probably due to selection of subpopulations of tumors *in vitro*, as expressed by a change in DNA content in some of the cell lines after several passages in culture compared to the tumors from which they were initiated. Therefore, extrapolation of results of *in vitro* chemosensitivity testing with a similar model for individual patients as suggested by Carmichael (26), should be interpreted with caution.

REFERENCES

1. Ianuzzi M.C., Scoggin C.H. Small cell lung cancer, state of the art. Am. Rev. Resp. Dis. 134: 593-608, 1986.
2. Hansen H.H., Rorth M., Aisner. Managment of small - cell carcinoma of the lung. In: Lung cancer, contemporary issues in clinical oncology, Aisner J. (ed), Churchill Livingstone, New York, 1985.
3. Goldie J.H., Goldman A.J. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. Cancer Res. 44:3643-3653, 1984.
4. de Leij L.F.H.M, Postmus P.E., Buys C.H.C.M., Elema J.D., Ramaekers F., Poppema S., Brouwer M., van der Veen A.Y., Mesander G., The T.H. Characterization of three new variant type cell lines from small cell carcinoma of the lung. Cancer Res. 45:6024-6033, 1985.
5. Berendsen H.H., de Leij L.F.H.M., de Vries E.G.E., Mesander G., Mulder N.H., de Jong B., Postmus P.E., Poppema S., Sluiter H.J., The T.H. Characterization of three small cell lung cancer cell lines established from one patients during longitudinal follow-up. Cancer Res. 48:6891-6899, 1988.
6. de Vries E.G.E., Meijer C., Timmer-Bosscha H., Berendsen H.H., de Leij L.F.H.M., Scheper R.J., Mulder N.H. Resistance mechanisms in three human small cell lung cancer cell lines established from one patient during clinical follow-up. Cancer Res. 49:4175-4178, 1989.
7. Minna J.D., Carney D.N., Oie H., Bunn P.A., Gazdar A.F. Growth of human small cell lung cancer cells in defined medium. In: A. Pardee, G Sato eds. Growth of cell in defined medium, Cold Spring Harbour, NY: Cold Spring Harbour Press, 629-639, 1982.
8. Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65:55-63, 1983.
9. Carmichael J., DeGraff W.G., Gazdar A.F., Minna J.D., Mitchell J.B. Evaluation of a Tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res. 47:936-942, 1987.
10. Timmer-Bosscha H., Hospers G.A., Meijer C., Mulder N.H., Muskiet F.A., Martini J.A., Uges D.R., de Vries E.G.E. The influence of docosahexaenoic acid on cisplatin resistance in a human small cell lung carcinoma cell line. J. Natl. Cancer Inst. 81:1069-1074, 1989.
11. Ruckdeschel J.C., Carney D.N., Oie H.K., Russell E.K., Gazdar A.F. in vitro chemosensitivity of human lung cancer cell lines. Cancer Treat. Rep. 71:697-704, 1987.
12. Bachur N.R., Moore A.L., Bernstein J.G., Lin A. Tissue distribution and disposition of daunomycin in mice, fluorometric and isotopic methods. Cancer Chemother. Rep. 54:89-94, 1970.
13. de Leij L.F.H.M., Poppema S., Klein Nuland J., ter Haar J.G., Scwander E., The T.H. Immunoperoxidase staining on frozen tissue sections as a first screening assay in the preparation of monoclonal antibodies directed against SCLC. Eur J. Cancer Clin. Oncol. 20:123-128, 1984.
14. de Leij L.F.H.M., Postmus P.E., Poppema S., Elema J.D., The T.H. The use of monoclonal antibodies for the pathological diagnosis of lung cancer. In: Hansen H.H. (ed), Lung cancer: Basic and clinical aspects, Martinus Nijhoff Publishers, Boston, 31-48, 1986.
15. Postmus P.E., Hirschler-Schulte C.J.W., de Ley L.F.H.M., Poppema S., Elema J.D., Edens E.T., Mesander G., Sluiter H.J., The T.H. Diagnostical application of a monoclonal antibody against small cell lung cancer. Cancer 57:60-63, 1986.
16. de Leij L.F.H.M., Poppema S., Klein-Nuland J. ter Haar A., Scwander E., Ebbens F., Postmus P.E., The T.H. Neuroendocriene differentiation antigen on human lung carcinoma and Kulchitski cells. Cancer Res. 45:2192-2200, 1985.
17. Moll R., Franke W.W., Schiller D.L., Geiger B., Krepler R. The catalogue of human cytokeratin polypeptides: patterns of expression of cytokeratins in normal epithelia, tumors and cultured cells. Cell 31:11-24, 1982.

18. Broers J.L.V., Carney D.N., de Ley L.F.H.M., Vooijs G.P., Ramaekers F.C.S. Differential expression of intermediate filament proteins distinguishes classic from variant small cell lung cancer cell lines. Proc. Natl. Acad. Sci. USA 82:4409-4413, 1985.

19. Ramaekers F., Hijsmans A., Moesker O., Kant A., Jap P., Herman C., Vooijs G. Monoclonal antibodies to keratin filaments, specific for glandular epithelia and their tumors. Use in surgical pathology. Lab. Invest. 49:353-361, 1983.

20. Kluk P., van Muyen G.N.P., van der Kamp A.W.M., Tibboel D., van Hoorn W.A., Warnaar S.O., Molenaar J.C. Hirschprung's disease studied with monoclonal antineurofilament antibodies on tissue sections. Lancet i:652-654, 1984.

21. Ruff M.R., Farrar W.L., Pert C.B. Interferon γ and granulocyte/macrophage colony stimulating factor inhibit growth and induce antigens characteristic of myeloid differentiation in small cell lung cancer cell lines. Proc. Natl. Acad. Sci. USA 83:6613-6617, 1986.

22. Juliano R.L., Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochem. Biophys. Acta 455:152-162, 1976.

23. Scheper R.J., Bulte J.W.M., Brakkee J.G.P., Quak J.J., van der Schoot E., Balm A.J.M., Meijer C.J.L.M., Broxterman H.J., Kuiper C.M., Lankelma J., Pinedo H.M. Monoclonal antibody JSB-1 detects a highly conserved epitope on the P-Glycoprotein associated with multidrug resistance. Int. J. Cancer 42:389-394, 1988.

24. Vindelov L.L., Christensen Ib.J., Nissen N.I. A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry 3:323-327, 1983.

25. Vindelov L.L., Christensen Ib.J., Nissen N.I. Standardization of high resolution flow cytometric DNA analysis by the simultaneous use of chicken and trout red blood cells as internal reference standards. Cytometry 3:328-331, 1983.

26. Carmichael J., Mitchell J.B., DeGraff W.G., Gamson J., Gazdar A.F., Johnson B.E., Glatstein E., Minna J.D. Chemosensitivity testing of human lung cancer cell lines using the MTT assay. Br. J. Cancer 57:540-547, 1985.

27. Cole S.P.C. Rapid chemosensitivity testing of human lung tumour cells using the MTT assay. Cancer Chemother. Pharmacol. 17:259-263, 1986.

28. Hill B.T. Use of continuous human tumour cell lines to evaluate drugs by clonogenic assays. In: Dendy P.P., Hill B.T. (eds) Human tumour drug sensitivity testing *in vitro*, Academic Press, London, 129, 1983.

29. Ling V. Does P-Glycoprotein predict response to chemotherapy? J. Nat. Cancer Inst. 81:84-85, 1989.

30. Fojo A.T., Akiyama S.I., Gottesman M.M. Reduced drug accumulation in multiple drug-resistant human KB carcinoma cell lines. Cancer Res. 45:3002-3007, 1985.

31. Goldstein L.J., Galski H., Fojo A., Willingham M., Shinn-Liang L., Gazdar A., Pirker R., Green A., Crist W., Brodeur G.M., Lieber M., Cossman J., Gottesman M.M., Pastan I. Expression of a multidrug resistance gene in human cancers. J. Natl. Cancer Inst. 81:116-124, 1989.

32. de Jong S., Zijlstra J.G., de Vries E.G.E., Mulder N.H. Reduced DNA topoisomerase II activity and drug induced DNA cleavage activity in an adriamycin resistant human small cell lung carcinoma cell line. Cancer Res. -Submitted.

33. Hospers G.A.P., Mulder N.H., de Jong B., de Leij L.F.H.M., Uges D.R.A., Fichtinger-Schepman A.M.J., Scheper R.J., de Vries E.G.E. Characterization of a human small cell lung carcinoma cell line with acquired cisplatin resistance *in vitro*. Cancer Res. -in press.

34. Schuurhuis G.J., Pinedo H.M., Cervantes A., Broxterman H.J., van Kalken C.K., van Heijningen Th. H.M., Lankelma J. Mechanism of anthracycline resistance and its reversal in cells with high and low levels of multidrug resistance. Proc. AACR, 1989, 2064.

35. Deffie A.M., Alam T., Seneviratne C., Beenken S.M., Batra J.K., Shea T.C., Henner W.D., Goldenberg G.J. Multifactorial resistance to Adriamycin: relationship of DNA repair, Glutathione transferase activity, drug efflux, and P-Glycoprotein in cloned cell lines of Adriamycin-sensitive and -resistant P388 Leukemia. Cancer Res. 48:3595-3602, 1988.

36. Zijlstra J.G., de Vries E.G.E., Mulder N.H. Multifactorial drug resistance in an adriamycin resistant human small cell lung carcinoma cell line. Cancer Res. 47:1780-1784, 1987.
37. Berry R.J., Laing A.H., Wills J. Fresh explant culture of human tumors *in vitro* and the assessment of sensitivity to cytotoxic chemotherapy. Br. J. Cancer 31:218-227, 1975.
38. Engelholm S.A., Vindelov L.L., Spang-Thomson M., Brunner N., Tommerup N., Nielsen M.H., Hansen H.H. Genetic instability of cell lines derived from a single human small cell carcinoma of the lung. Eur. J. Cancer Clin. Oncol. 21:815-824, 1985.
39. Bunn P.A., Carney D.N., Gazdar A.F., Wang-Pheng J., Matthews M.J. Diagnostic and biological implications of flow cytometric DNA content analysis in lung cancer. Cancer Res. 43:5026-5032, 1983.
40. Gazdar A.F., Minna J.D. Cell lines as an investigational tool for the study of biology of small cell lung cancer. Eur. J. Cancer Clin. Oncol. 22:909-911, 1986.
41. Berendsen H.H., de Leij L.F.H.M., Postmus P.E., Poppema S., Boes A., Sluiter H.J., The T.H. Neuroendocrine differentiation antigens and response to chemotherapy in small cell lung cancer. Thesis, Groningen, pp 73-82, 1988.

Chapter 11

LIMITATIONS OF THE FAST GREEN ASSAY FOR CHEMOSENSITIVITY TESTING IN HUMAN LUNG CANCER

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SUMMARY

Selection of patients with lung cancer who are most likely to benefit from chemotherapeutic treatment would be a substantial step forward. Therefore, a prospective study in predictive chemosensitivity testing in vitro using the fast green assay (FGA) as developed by Weisenthal et al. was carried out. Sixty-six pretreatment tumor specimen were obtained; the majority by means of bronchoscopy (n=42). Due to an initially insufficient yield of tumor cells (n=19), dead cells in control samples after 4-day culture (n=15), contamination (n=7) and laboratory failure (n=2), only 23 (38.9%) samples were successfully tested. In 14/36 patients a comparison between in vitro and in vivo response was possible. Taking into account the number of failures, this number of successful assays does not allow for any conclusion regarding accuracy of the FGA. We conclude that the FGA has limited usefulness in in vitro chemosensitivity testing of human bronchogenic cancer. Future directions for predictive testing in vitro are discussed

INTRODUCTION

The chemotherapeutic management of human lung cancer requires careful balancing between potential benefits and predictable side effects. Especially in non small cell lung cancer (NSCLC) this is obvious, because most chemotherapy regimen will produce responses in only a modest proportion of patients (1). Moreover, the effect on survival, also in those patients who are responding to chemotherapy, is limited. As a consequence the administration of cytotoxic drugs to patients suffering from this malignancy on a trial and error base is questioned by several authors (2-4). In small cell lung cancer (SCLC) most patients show impressive responses to combination chemotherapy regimen (5). However, in the vast majority of patients the tumor relapses, and not all patients will benefit from second line treatment (6). Thus, selection of patients with inoperable lung cancer who are most likely to have any benefit of cytostatic treatment would constitute a substantial step forward. Therefore we performed a feasibility study for a prospective trial of pre-

dictive chemosensitivity testing on fresh tumor biopsy material in patients with lung cancer.

MATERIALS AND METHODS

Tumor cell preparation

Fresh human lung cancer material was processed as described in a previous report (7). Briefly, fresh human tumor specimen were placed in RPMI 1640 medium and 10% fetal calf serum (FCS) with penicillin (125 U/ml), streptomycin (125 μg/ml) and amphotericin B (1 μg/ml). If necessary the specimen were grossly minced with scalpels and scissors. Since it is unnecessary to obtain single cell suspensions for the dye exclusion assay (8), excessive trauma was avoided. The cell suspension was incubated with 0.1% collagenase and 0.003% DNase for at least 1 hour at 37°C. Cells from malignant effusions were pretreated with a buffer (8.29 g NH_4Cl, 1.0 g $KHCO_3$, 0.0372 g $Na_2 EDTA/L$ distilled water) in order to lyse red blood cells. Cells from bone marrow aspirates were collected in heparin (5000 U/L) containing tubes. The number of viable nucleated cells was quantified by means of trypan blue exclusion. Based on our previous experience a minimum number of 4×10^5 viable cells was considered necessary to perform a control and three drug treated cultures.

Drugs

Vincristine for infusion (Eli Lilly, Indianapolis, USA) was dissolved in phosphate buffered saline (PBS) and kept at 4 °C at twenty times the desired concentration for a maximum of two weeks. Carboplatin for infusion (Paraplatin, Bristol Myers, Weesp, The Netherlands) was dissolved in glucose 5% at a concentration of 10 mg/ml and kept at 4 °C for a maximum of 7 days.

Drug sensitivity assay

The fast green assay (FGA) as described by Weisenthal et al (8-11) was used with some minor modifications (7). The cells were resuspended in fresh Dulbecco's Modified Eagle's Medium (DME)/F_{12} (1:1) supplemented with 20% FCS with a minimum concentration of 10^5 cells/ml with 10^5 fixed chicken red blood cells as internal standard and incubated for 1 hour at 37 °C with vincristine 0.5 μg/ml, carboplatin 18.5 μg/ml and combination of these two drugs using the same concentrations. Untreated controls were incubated with the solvent used instead of the drug. After incubation, cells were washed three times with PBS and resuspended in 2.5 ml of fresh medium (DME/F_{12}, 20% FCS) in polypropylene tubes for a short term culture period of 4 days under a humified atmosphere of 95% air, 5% CO_2 at 37 °C. At day 4, cells were stained with 1% Fast Green for 10 min and sedimented onto microscope slides using a Cytospin centrifuge (550 rpm, 5 min) and counterstained with a modified haematoxylin-eosin technique. Under the microscope

dead cells stain green and the fixed chicken red blood cells which are oval and nucleated stain predominantly green. Living cells retain their characteristic appearance with hae-matoxylin-eosin. The fixation of the internal standard of the chicken red blood cells was performed as described before (7). A successful assay was defined as an assay in which at least 100 viable and recognizable neoplastic cells were present on the control slides and in which a result could be obtained for all drug treated samples (11). The ratio of living tumor cells over fixed chicken red blood cells was determined for each triplicate of cen-trifuge slide of drug treated cells. Results are expressed as percentage of untreated con-trols. A 30% cell survival was used as the cutoff between 'response' and 'resistant' *in vitro* (9).

RESULTS

In vitro chemosensitivity testing

Sixty-six pretreatment tumor specimen were obtained. Table I shows the origin and his-tology of the specimen. Most were obtained by fiberoptic bronchoscopy (n=20) or by rig-id bronchoscopy (n=22). In all patients at least one biopsy was taken for histopathological diagnostic procedures and as many as possible for predictive testing. Fifteen of the bron-choscopy specimen (13 SCLC) and 8 of otherwise obtained specimen (all SCLC) were taken from previously treated patients.

Table II shows the results of the FGA. Finally, 34.8 % (n=23) of the specimen were evaluable for chemosensitivity testing *in vitro*. (23.8% of the bronchoscopy specimen, 54.2% of the other specimen). The advantage of rigid bronchoscopy over fiberoptic bron-choscopy consists of the potential of the former method to obtain larger biopsies. This is immediately shown by the fact that failure to perform a FGA on fiberoptic bronchoscopy specimen due to an initial insufficient number of cells was encountered in 60% (n=12) of specimen (n=20). For the rigid bronchoscopy specimen this number was 22.7% (n=5). The median number of cells obtained with fiberoptic bronchoscopy was 1.5×10^5 (range $0-1.4 \times 10^6$), median viability 7%, and with rigid bronchoscopy 7.0×10^5 (range $2.7 \times 10^{4-}$ 9×10^6), median viability 16%. As in our previous study, a high percentage of cell death in control cultures after four days was found (table 2). In 25% (n=11) of the bronchoscopy

Table I.   Origin and histology of the tumor specimens.

	Number of specimens	SCLC	NSCLC
Rigid bronchoscopy	22	8	14
Flexible bronchoscopy	20	10	10
Lymph node metastases	11	9	2
Malignant effusions	8	5	3
Bone marrow aspirates	5	5	0
Total	66	37	29

specimen all cells in the control samples were dead after four days culture, which made drug evaluation impossible in these specimen. The mean number of cells obtained from the other specimen was 4.9 x 10^6 (range 4.4 x 10^4 - 4.3 x 10^7) with a viability above 10% (range 5-96%). In this group there were only two failures due to an initial insufficient number of cells. Three specimen were not evaluable due to infection and two due to laboratory failure. In four specimen all cells were dead after four days culture. These figures

Table II.   Results Fast Green Assay of tumor specimens.

	Bronchoscopy specimens	Other specimens
Number	42	4
Evaluable with FGA	10	13
Failure FGA due to:		
- initial insufficient number of cells	17	2
- contamination	4	3
- after 4 days all cells dead in control culture	11	4
- laboratory failure	0	2
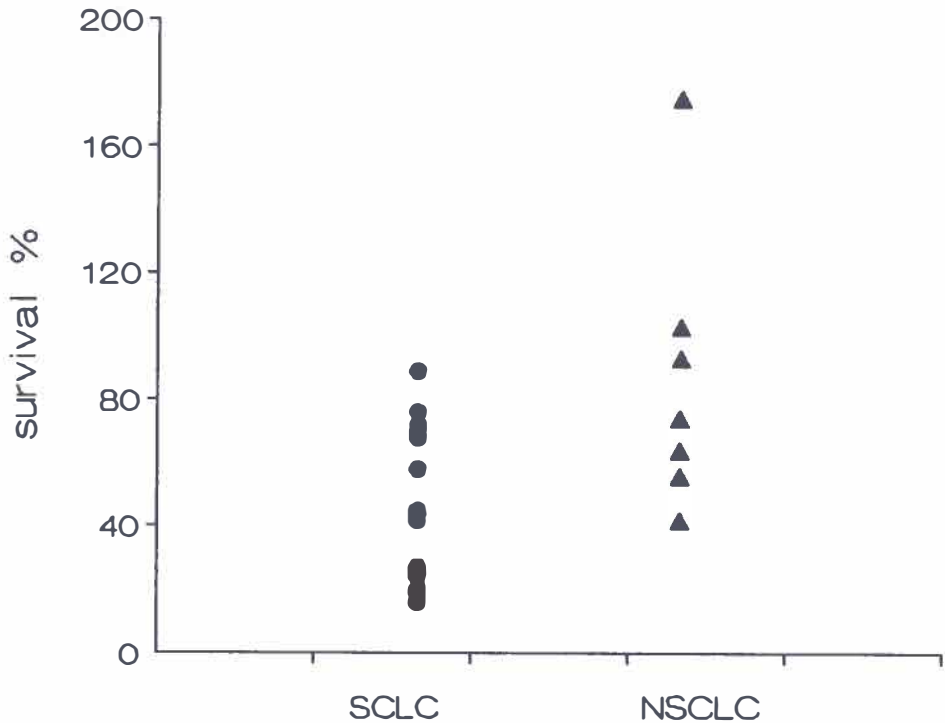
Figure 1.   Chemosensitivity for carboplatin (18.5 µg/ml for 1 hour) and vincristine (0.5 µg/ml for 1 hour) measured with FGA in human tumor specimen.

are somewhat higher compared to our previous study, in which there were no failures due
to an initially low number of cells or after the culture period in controls. Twenty-three spe-
cimen (7 NSCLC and 16 SCLC) of the total number of specimen obtained could be eva-
luated for chemosensitivity testing *in vitro* with the FGA.

Figure 1 shows the results of the FGA if evaluable. Of the SCLC specimen (n=16) 7
(43.7%) had less than 30% survival of viable tumor cells versus internal standard in the
FGA after incubation with the combination of carboplatin 18.5 µg/ml and vincristine 0.5
µg/ml (range 16-89%) (fig 1). For the individual drugs the median figures were 75% sur-
vival (range 6-132%) for carboplatin and 69.8% survival (range 20-157%) for vincristine
(not depicted). There was no evidence found of synergistic drug effects. None of the
NSCLC specimen evaluable *in vitro* (n=7) had over 70% reduction of viable tumor cells
versus internal standard (fig 1).

Comparison of in vivo with *in vitro* response (table III)
At the time of this study, patients with lung cancer were treated on various chemotherapy
protocols in our institution. The results of these studies are published elswhere (12,13).
Nineteen of the 37 patients with small cell lung cancer of whom tumor biopsy specimen
were obtained for *in vitro* chemosensitivity testing were treated with carboplatin (400
mg/m^2) and vincristine (2 mg days 1 and 8) every four weeks *in vivo*. Eight (42.5%) of
these specimen were evaluable for response. Six specimen were considered non-respon-
der and two were considered responder *in vitro*. These specimen were obtained from 8
SCLC patients of whom 4 showed a partial response (as defined by WHO criteria) after
two courses of carboplatin and vincristine and 4 had no clinical response.

Seventeen patients with non small cell lung cancer were treated with carboplatin and
vincristine in the same schedule as mentioned above. Of all these patients tumor biopsy
specimen were available for *in vitro* testing. Six specimen were evaluable for response *in
vitro*, none showed more than 70% reduction of viable cells versus internal standard. Of

Table III. Comparison of in vitro and in vivo response.

| | SCLC | | NSCLC | |
	in vitro	in vivo	in vitro	in vivo
Responder	2	4	0	0
Non responder	6	4	6	5[1]

[1] Two biopsy specimen originated from one patient.

the five patients of which these specimen originated three had progressive disease after
one course of carboplatin and vincristine chemotherapy and two patients had stable dise-
ase after two courses of this regimen.

Thus, ultimately in 14 out of 36 patients (38.9%) a comparison between *in vitro* and
in vivo treatment was possible. Taking into account the number of assay failures, the small

number of successful *in vitro* assays does not allow for analysis in terms of sensitivity or specificity of the FGA in this study.

DISCUSSION

There have been many attempts to develop tests to predict responses to anti-cancer drugs during the past forty years (14-17). Two important uses for such a test are evident. Firstly, it would be invaluable for screening new compounds and drug regimen for potential use in treating patients with cancer. Secondly, with pretreatment testing of a patient's tumor against a wide range of agents it might be possible to tailor a patient's treatment individually. Despite the many proposed tests, none has achieved wide-spread acceptance. Mattern et al. (18) reviewed the ideal prerequisites of such a test: it should be technically simple, fast, inexpensive, reproducible and applicable for all tumors. In addition the test should be capable of taking into account the various methods of drug action and be able to predict with reasonable accuracy the clinical results. In this study the FGA developed by Weisenthal and coworkers was used. This assay meets some of the above mentioned criteria for the 'ideal' *in vitro* chemosensitivity assay. However, the limitations of the FGA in bronchogenic cancers to this end are demonstrated by this study. In lung cancer, bronchoscopy is often the easiest way to obtain tumor material. A major obstacle for successful *in vitro* testing with this material was the initially low yield of viable tumor cells. Secondly, in the majority of specimen left, poor survival of the neoplastic cells in control cultures was the most common reason for failure assays. These problems were recognized by Weisenthal et al. when testing human lymphatic neoplasms *in vitro* (11). In our previous study of *in vitro* chemosensitivity testing of human bronchoscopy specimen (7) only 25% of the biopsies could be successfully tested with the FGA. Failures were again due to the same reasons as mentioned above. Several authors have suggested that the ability of tumor cells to survive *in vitro* may be of negative prognostic importance (19-21). Thus, one might argue that the low number of successful assays might be a reflection of the low number of therapy resistant patients included in this trial. However, in a recent report, Stevenson et al. (23) found no significant survival differences in a group of 68 SCLC patients between those whose tumor cell specimen grew in culture versus the others. Also, as carboplatin and vincristine produce a 16% response rate in NSCLC (Smit et al. manuscript in preparation), the number of assay failures (12/19) in this group are most probably due to the assay itself. In other than bronchoscopy specimen assay failure due to initially low number of cells was encountered only twice and no living cells after four days culture in 4 cases. The 55% success rate in these specimen is lower than the 75% success rate with these specimen in our earlier study. The number of viable cells obtained (median 4.9×10^6) would have allowed for clonogenic assays as about 5×10^5 per dish are needed. However, when data from 6 studies using lung cancer specimen in the clonogenic assay are pooled they show about 60% successful clonogenic assays (>30 colonies/dish) (24-29). The FGA is easier to perform and faster. In addition several authors have shown that results obtained

with the latter assay show good correlation with the clonogenic assay. Therefore, in situations where a sufficient number of cells is available for drug testing the FGA may have advantages over the clonogenic assay.

In the study under discussion the FGA was used in a prospective setting. The low number of successful assays does not allow for any statement in regard to the accuracy of this test. Weisenthal et al. communicated a prospective trial in lymphatic malignancies using the FGA (12). Their results showed significant correlations (p<0.0001) with the clinical response obtained in 69 patients with the same drugs as used *in vitro*.

A way to circumvent some of the problems encountered in this study may be to investigate specific properties of tumor cells thought to be associated with drug resistance rather than looking at cell kill *in vitro*. Recently, in three reports it was shown that expression of neuroendocrine antigens on lung cancer cells correlate with survival (30) and response to chemotherapy (31,32). Salmon et al. (33) saw significantly less responses to doxorubicin *in vitro* in myeloma, lymphoma and breast cancer specimen whose tumor cells expressed the P-glycoprotein. Refinement of molecular biological techniques should make it possible to study in the future the cellular localization and quantitation of expression of genes and gene products related to resistance in individual cells in tumor specimen (34). Alternatively, assays such as anthracycline accumulation (35,36) may help to predict whether resistance mechanisms are functionally present.

We may conclude that the FGA has limited usefulness in *in vitro* chemosensitivity testing of human bronchogenic cancer. Assay failures are largely due to low yield of tumor cells by bronchoscopy and the low number of tumor cells surviving four days *in vitro*. Due to these and other basic problems associated with predictive testing, a reliable and accurate predictive test remains a rather distant objective (37).

REFERENCES

1. Minna JD, Pass H, Glatstein E, Ihde DC. Cancer of the lung. In: DeVita VT, Hellman S, Rosenberg SA (eds). Cancer, principles and practice of oncology 3ed, J.B. Lippincott Co., Philadelphia, 1989, pp 659-664.
2. Williams CJ, Woods R, Levi J, Page J. Chemotherapy for non-small cell lung cancer: a randomized trial of cisplatin/vindesine vs no chemotherapy. Seminars in Oncology 15:58-61, 1988 (suppl 7).
3. Ganz PA, Figlin RA, Haskell CM, La Soto N, Siau J. Supportive care versus supportive care and combination chemotherapy in metastatic non-small cell lung cancer. Does chemotherapy make a difference? Cancer 63:1271-1278, 1989.
4. Rapp E, Pater JL, Willan A. Chemotherapy can prolong survival in patients with advanced non-small cell lung cancer-Report of a canadian multicenter randomized trial. J. Clin. Oncol. 6:633-641, 1988.
5. Ihde DC. Current status of therapy for small cell carcinoma of the lung. Cancer 1985; 54: 2722-2728.
6. Postmus PE, Berendsen HH, van Zandwijk N, Splinter TAW, Burghouts JThM, Bakker W, the EORTC Lung Cancer Cooperative Group. Retreatment with the induction regimen in small cell lung cancer relapsing after an initial response to short term chemotherapy. Eur. J. Cancer Clin. Oncol. 1987, 23, 1409-1411.

7. De Vries EGE, Meijer C, Mulder NH, Postmus PE. *in vitro* chemosensitivity testing of human lung cancer for vindesine. Eur. J. Cancer Clin. Oncol. 23:55-60, 1987.

8. Weisenthal LM, Dill PL, Kurnick NB, Lippman ME. Comparison of dye exclusion assays with a clonogenic assay in the determination of drug-induced toxicity. Cancer Res. 43:258-264, 1983.

9. Weisenthal LM, Marsden JA, Dill PL, Macaluso CK. A novel dye exclusion method for testing *in vitro* chemosensitivity of human tumors. Cancer Res. 43:749-757, 1983.

10. Weisenthal LM, O'Tayo Lalude A, Miller JB. *in vitro* chemosensitivity of human bladder cancer. Cancer 51:1490-1496, 1983.

11. Weisenthal LM, Dill PL, Finklestein JZ, Duarte TE, Baker JA, Moran EM Laboratory detection of primary and aquired drug resistance in human lymphatic neoplasms. Cancer Treat. Rep. 70:1283-1295, 1986.

12. Postmus PE, Kirkpatrick A, Dalesio O, Palmen FJ, Carney DN, Festen J, Vendrik C, Roozendaal K, Burghouts J, Planting A, Quoix E, Splinter TAW, McVie JG. Two carboplatin-containing regimen for small cell lung cancer: preliminary results of a randomized phase II trial. Cancer Treatment Reviews 15:41-44, 1988 (Suppl.B)

13. Smit EF, Berendsen HH, de Vries EGE, Mulder NH, Postmus PE. A Phase II study of carboplatin and vincristine in previously treated patients with small cell lung cancer. Cancer Chemother. Pharmacol. 25: 202-204, 1989.

14. Black MM, Speer FD. Further observations on the effects of cancer chemotherapeutic agents on the *in vitro* dehydrogenase activity of cancer tissue. J. Natl. Cancer Inst. 14:1147-1158, 1954.

15. Hanauske AR, Hanauske U, Von Hoff DD. The human tumor cloning aasay in cancer research and therapy: a review with clinical correlations. Curr. Probl. Cancer 9:1-50, 1985.

16. Tannenberger S, Nissen E. Predicting response of human solid tumors to chemotherapy. Cancer Treatment Reviews 10:203-219, 1983.

17. Weisenthal LM, Su Y, Duarte TE, Nagourney RA. Non-clonogenic *in vitro* assays for predicting sensitivity to cancer chemotherapy. In: Hall T.C. (ed) Prediction of response to cancer therapy. Progress in clinical and biological research, vol. 276, Alan R. Liss, Inc., New York, 1988.

18. Mattern J, Volm M. Clinical relevance of predictive tests for cancer chemotherapy. Cancer Treatment Reviews 9:267-298, 1982.

19. Reynolds CP, Frenkel EP, Smith RG. Growth characteristics of neuroblastoma *in vitro* correlate with patient survival. Trans Assoc. Am. Physicians 93:203-211, 1980.

20. Miller OL, Grover FL, Trinkle JK. Growth of tumor colonies in soft agar is a prognostic factor for patients with lung cancer. Surg. Oncol. 30:435-437, 1984.

21. Bertoncello I, Bradly TR, Campbell JJ, Day AJ, McDonald IA, McLeish GR, Quinn MA, Rome R, Hodgson GS. Limitations of the clonal agar assay for the assessment of primary human ovarian tumour biopsies. Br. J. Cancer 45:803-811, 1982.

22. Johnson BE, Ihde DC, Makuch RW. Myc family oncogene amplification in tumor cell lines established from small cell lung cancer patients and its relationship to clinical status and outcome. J. Clin. Invest. 79:1629-1634, 1987.

23. Stevenson HC, Gazdar AF, Linnolia RI, Russel EK, Oie HK, Steinberg SM, Ihde DC. Lack of relationship bbetween *in vitro* tumor cell growth and prognosis in extensive stage small cell lung cancer. J. Clin. Oncol. 7:923-931, 1989.

24. Von Hoff DD, Casper J, Bradley E, Sandbach J, Jones D, Makuch R. Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. Am. J. Med. 70:1027-1032, 1981.

25. Von Hoff DD, Coltman CA, Forseth B. Activity of 9-10 anthracenedicarboxaldehyde bis[(4, 5-dihydro-1 H-imidazol2-yl) hydrazone] dihydrochloride (CL 216, 942) in a human tumor cloning system. Cancer Chemother. Pharmacol. 6:141-144, 1981.

26. Salmon SE, Lin RM, Casazza AM. Evaluation of new anthracycline analogs with the human tumor stem cell assay. Cancer Chemother. Pharmacol. 6:103-110, 1981.
27. Pavelic ZP, Slocum HK, Rustum YM, Creaven PJ, Nowak NJ, Karakousis C, Takita H, Mittelman A. Growth of cell colonies in soft agar from biopsies of different human solid tumors. Cancer Res. 40:4151-4158, 1980.
28. Von Hoff DD, Cowan J, Harris G, Reisdorf G. Human tumor cloning: feasibility and clinical correlations. Cancer Chemother. Pharmacol. 6:265-271, 1981.
29. Von Hoff DD, Clark GM, Stogdill BJ, Sarosdy MF, O'Brien MT, Casper JT, Mattox DE, Page CP, Cruz AB, Sandbach JF. Prospective clinical trial of a human tumor cloning system. Cancer Res. 43:1926-1931, 1983.
30. Berendsen HH, de Leij L, Poppema S, Postmus PE, Boes A, Sluiter HJ, The H. Clinical characterization of non-small cell lung cancer tumors showing neuroendocrine differentiation features. J. Clin. Oncol 7:1614-1620, 1989.
31. Graziano SL, Mazid R, Newman N, Tatum A, Oler A, Mortimer JA, Gullo JJ, DiFino SM, Scalzo AJ. The use of neuroendocrine immunoperoxidase markers to predict chemotherapy response in patients with non-small cell lung cancer. J. Clin. Oncol 7:1398-1406, 1989.
32. Berendsen HH, de Leij L, Postmus PE. Tumor cell phenotype detected by monoclonal antibodies and response to chemotherapy in small cell lung cancer. Chest 91:11-12, 1987.
33. Salmon SE, Grogan TM, Miller T, Scheper R, Dalton WS. Prediction of resistance *in vitro* in myeloma, lymphoma and breast cancer by P-glycoprotein staining. J. Natl. Cancer Inst. 81:696-701, 1989.
34. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A, Pirker R, Green A, Crist W, Broder GM, Lieber M, Cossman J, Gottesman MM, Pastan I. Expression of a multidrug resistant gene in human cancers. J. Natl. Cancer Inst. 81:116-124, 1989.
35. Nooter K, Herweyer H, Sonneveld P, Verwey J, Hagenbeek A, Stoter G. Detection of multidrug resistance in refractory canncer patients. Proc. AACR. 30:2092, 1989.
36. Marayana Y, Murohashi I, Nara N, Aoki N. Effects of verapamil on the cellular accumulation of daunorubicin in blast cells and on the chemosensitivity of leukaemic blast progenitors in acute meyelogenous leukaemia. Br J Haematol 72:357-362, 1989.
37. Twentyman PR. Predictive chemosensitivity testing. Br. J. Cancer 51:295-299, 1985.

Chapter 12

SUMMARY AND CONCLUSIONS

In this thesis several aspects of palliative chemotherapy for lung cancer patients are described. The general outlines of the thesis are described in the introduction.

In chapter 1 a review is given of factors influencing treatment outcome in elderly patients (>70 years of age) with SCLC. Due to the increasing proportion of the elderly in the population as a whole there will be an increasing number of elderly patients presenting with SCLC in the near future. Several factors are responsible for the fact that elderly persons with SCLC are not treated with the same intent as younger persons are. However, based on the available data in the literature, it is not certain that high age alone is an adverse prognostic factor neither in terms of response to chemotherapy, nor in terms of survival or toxicity of this type of treatment.

Chapter 2 contains the results of a phase II study of orally administered etoposide, total dose 800 mg/m^2 divided over 5 consecutive days every 4 weeks, in elderly patients (>70 years) with SCLC. In 35 previously untreated patients an overall response rate of 71% was observed, with a median survival of 16 months for patients with limited disease and 9 months for those with extensive disease. These figures are comparable to results obtained with multidrug regimen in younger persons, especially with regard to survival. However, toxicity of the investigated regimen was considerably lower. It is concluded that orally administered etoposide in this schedule is a suitable regimen for palliation of elderly SCLC patients.

In chapter 3 a phase I study with orally administered teniposide, the congener of etoposide, is described. This drug may be even more effective against SCLC than etoposide, but has not yet been administered in this way. The optimal dose for phase II studies was found to be 135 mg/m^2 (orally administered) once daily x 5, every 3 weeks. The dose limiting toxicity was both haematological and gastro-intestinal (nausea and vomiting, diarrhea). The latter toxicity may be resolved by substituting the intravenous ampoules used in this study by capsules. Until this method of oral administration has been developed, studies with oral teniposide are not recommended.

In chapter 4 the results of a phase II study in patients with symptomatic central nervous metastases of SCLC are reported. Patients were treated with teniposide 150 mg/m^2 (i.v.) x 3 every 3 weeks for a maximum of 12 cycles. In 11 out of 26 evaluable patients a response was seen. Median response duration was 42 weeks. Toxicity was acceptable, WHO grade 3/4 leukocytopenia was seen in 37% and thrombocytopenia in 16% of 123 courses. We conclude that teniposide is active against brain metastases of SCLC and is a suitable drug for palliation of these patients.

In chapter 5 and 5[a] the recently developed second generation platinum compound carboplatin was evaluated for its nephrotoxicity. Carboplatin has been developed in

search of a cisplatin analogue with comparable antitumor efficacy but with reduced toxicity, especially nephrotoxicity. The latter toxicity limits the clinical usefulness of cisplatin. Renal function was prospectively determined in 10 untreated patients with lung cancer with normal baseline renal function, treated with carboplatin 400 mg/m^2 and vincristine 2 mg (days 1 and 8) every 4 weeks. After the second course a significant fall in GFR and ERPF started, ultimately leading to a median decrease in GFR of 19.0% and in ERPF of 14%. No increases in the excretion of tubular enzymes or changes in the relative ß$_2$-microglobulin clearances were seen. We conclude from our data that carboplatin causes loss of renal function. As in individual patients no excessive myelosuppression was observed in conjunction with loss of renal function, the clinical relevance of our findings is not clear.

In the chapters 6 and 7 the efficacy of the combination of carboplatin (400 mg/m^2) and vincristine (2 mg, days 1 and 8) on a four week schedule in lung cancer patients was evaluated. In pretreated patients with SCLC (chapter 6) this regimen produced a response rate of 37% (10 out of 28), all partial responses. Of particular interest were the responses observed in a subgroup of patients relapsing within three months off induction therapy. These patients are considered clinically resistant to drugs used in the induction regimen. Therefore the responses seen in this group (8 out of 22) may be due to absence of cross resistance between the regimen used. The difference in responsiveness to chemotherapy between SCLC and NSCLC was demonstrated in chapter 7. Using the same regimen a 17% response rate in previously untreated patients with NSCLC was seen. Toxicity of the carboplatin vincristine combination differed somewhat in the two studies. In the SCLC group myelosuppression was the most frequent encountered toxicity; 26% of courses were associated with WHO grade III/IV myelosuppression, whereas in the NSCLC patients this was seen in only 4%. For nausea and vomiting (WHO grade II/III) these figures were 16% and 63% respectively. In both studies peripheral neuropathy due to vincristine was seen in about half of the patients. It is concluded that the role of carboplatin and vincristine in NSCLC remains to be defined.

Continuous infusion may be a method to improve the therapeutic index of cytotoxic drugs, by increasing the length of exposure of metabolically active tumor cells to the drug and simultaneously decrease toxicity related to high peak levels of the drug. In chapter 8 a phase I and pharmacokinetic study with 21 days continuous infusion of carboplatin is described. The maximum tolerable dose was found to be 30 mg/m^2/day for 21 days on a 6 week schedule. Haematological and non-haematological toxicities at this dose level were considerably lower compared to literature data of the same total dose (630 mg/m2) of carboplatin when given as a bolus infusion. Also, renal toxicity was reduced compared to bolus administration of carboplatin (chapter 5). Plasma pharmacokinetics of platinum were studied by flameless atomic absorption assay. Plasma steady state levels of free platinum were reached after 8 hours. During steady state, carboplatin dose and free platinum plasma levels were not correlated. For total platinum steady state serum levels were not reached. There was a significant relation

between total platinum serum levels and the drug dose administered. Significant relationships between glomerular filtration rate and steady state plasma levels of free platinum and percent reduction in platelet counts could be established. It is concluded that the pharmacokinetics of carboplatin are not significantly altered by this method of administration compared to literature data concerning bolus infusion of carboplatin. Immunohistochemical analysis of DNA bound platinum (the supposed cytotoxic lesion of platinum compounds) in leukocytes showed a linear increase between DNA bound platinum (platinum-DNA adducts) and duration of infusion in individual patients. There was no correlation between free platinum exposure and platinum-DNA adduct level. Noteworthy, one complete response and four partial responses in 17 (out of a total of 44) evaluable patients were observed.

In chapter 9 a phase I and pharmacokinetic study of continuous infusion of vindesine for 21 days is described. For vindesine, in contrast to other vinca alkaloids, it was not possible to enhance the therapeutic index by prolonging the infusion period. The study was aborted at the lowest dose level (0.2 mg/m^2/day) due to reversible but severe neurotoxicity in 3 out of 4 patients. The total dose of vindesine administered to these patients does usually not lead to neurotoxicity when given as a bolus infusion. No accumulation of vindesine in plasma during the infusion period was found. We conclude that due to this toxicity further evaluation of vindesine in this schedule is not recommended.

In chapter 10 the response of SCLC cell lines to cytotoxic drugs was correlated to the *in vivo* response to the same drugs in the patients the cell lines were derived from. We investigated whether the properties of cell lines obtained from patients who showed a clinical complete response to chemotherapy and of those obtained from patients who had no response were retained *in vitro*. When this assumption is proved to be correct, cell lines might be a valuable tool in predictive chemosensitivity testing. Using the micro titerwell tetrazolium assay, no correlation could be found. Next, 5 out of the 6 cell lines were compared to the tumor biopsies from which they were initiated. Using a panel of monoclonal antibodies, there were only minor differences between the tumor cells in the biopsies and in the cell lines. No conclusive evidence could be found for the existence of the PDR phenotype. In the 2 biopsies evaluable for DNA ploidy differences could be demonstrated compared to the corresponding cell lines. We concluded that the observed lack of correlation between the *in vitro* and *in vivo* response to cytotoxic drugs is due to selection of tumor cell populations in culture. Nevertheless, cell lines remain a invaluable instrument to study resistance mechanisms in lung cancer.

In chapter 11 a prospective study in predictive chemosensitivity testing on fresh tumor biopsy material using a dye exclusion assay (fast green assay) is described. The patients participating in this study were described in chapters 6 and 7. Out of 66 tumor biopsies 23 could successfully be tested in the fast green assay. Failures were in the majority due to an insufficient yield of tumor cells obtained with bronchoscopy and death of all tumor cells in the control cultures. It is concluded that the fast green assay has limited usefulness in predictive chemosensitivity testing in human lung cancer.

We conclude that results of palliative chemotherapy for patients with lung cancer can be improved in various ways. Elderly patients with small cell lung cancer can be treated with a simple and relatively non-toxic chemotherapy regimen. Patients with brain metastases from small cell lung cancer can be treated with chemotherapy alone. Future clinical trials should focus on the value of radiotherapy alone or in addition to chemotherapy in this situation.

Carboplatin is an active drug in small cell lung cancer. Its potential as a clinically non cross resistant drug with some of the frequently used agents in small cell lung cancer deserves further investigation. Continuous instead of bolus infusion of this drug may be advantageous as far as bone marrow toxicity and renal toxicity is concerned. Such advantages, and a comparison of antitumor effects should be studied further in a randomized way.

Further improvement in the fate of patients with lung cancer awaits new treatment strategies. Of critical importance will be the *in vitro* and *in vivo* investigation of drug resistance mechanisms - intrinsic and acquired - and their circumvention. New diagnostic tools such as offered by immunological and molecular biological techniques may give the opportunities to study these phenomena in human tumor material at the cellular level. Also, assays need to be developed to predict whether the supposed resistance mechanisms are present and functional in human tumors. Treatment policies based on results of these techniques will have to be developed. Only then will the main goal of therapy for lung cancer patients consist of both an improvement in their quality of life, and a substantial prolongation of survival or even cure.

Chapter 13

SAMENVATTING EN CONCLUSIES

Longkanker is de meest voorkomende aan kanker gerelateerde doodsoorzaak in de mannelijke populatie in Nederland; bij vrouwen komt deze op de derde plaats, na borstkanker en kanker van de dikke darm. Op het moment dat longkanker klinisch manifest wordt, kan slechts 10% genezen worden. Ondanks veel onderzoek is dit percentage al sinds geruime tijd niet toegenomen. Jaarlijks overlijden in Nederland ongeveer 8500 personen aan longkanker.

Longkanker wordt vanwege de therapiekeuze onderverdeeld in niet-kleincellige bronchuscarcinomen en kleincellige bronchuscarcinomen. Voor de niet-kleincellige bronchuscarcinomen vormt operatieve behandeling de enige kans op curatie indien het patiënten betreft zonder aantoonbare metastasen op afstand. Twintig tot 25% van de patiënten met longkanker heeft een kleincellig anaplastisch bronchuscarcinoom. Deze vorm van longkanker wordt bij voorkeur behandeld met een cytostatische therapie omdat er vrijwel altijd metastasen aanwezig zijn. Voor een klein aantal patiënten kan deze behandeling resulteren in genezing, meestal is er echter slechts een tijdelijke verbetering mogelijk van de kwaliteit van het leven. Het niet-kleincellig bronchuscarcinoom reageert doorgaans slecht op chemotherapie.

Het feit dat chemotherapie vaak niet leidt tot curatie is in essentie een probleem van resistentie. In de laatste jaren is een groot aantal resistentie mechanismen van tumorcellen tegen cytotoxische medicamenten in het laboratorium ontdekt. De meest bestudeerde vorm van resistentie is de zogenaamde pleiotropic drug resistance. Deze vorm van resistentie, die mogelijk wel te antagoneren is, lijkt echter geen rol van betekenis te spelen bij het bronchuscarcinoom. Van de andere resistentie-mechanismen is het nog volstrekt onduidelijk of zij in de nabije toekomst in de kliniek geantagoneerd kunnen worden. De vraag dringt zich dus op hoe het lot van patiënten met een gemetastaseerd bronchuscarcinoom verbeterd kan worden. Vooralsnog lijkt intensivering van chemotherapie, eventueel met behulp van hematopoietische groeifactoren, de enige manier om de resultaten op korte termijn te verbeteren. Echter, slechts een kleine groep patiënten zal hiervoor in aanmerking komen, gezien de onvermijdelijke emstige toxiciteit van deze behandeling. Voor de meerderheid van de patiënten met een bronchuscarcinoom zal dus verbetering gezocht moeten worden in het verminderen van de toxiciteit van de behandeling, idealiter met behoud van de resultaten met betrekking tot tumor remissie en overleving zoals die heden ten dage bereikt worden. In dit proefschrift worden verschillende aspecten van palliatieve chemotherapie voor het longcarcinoom beschreven.

In hoofdstuk 1 wordt een overzicht gegeven van de factoren die van invloed zijn op de resultaten van behandeling met cytotoxische medicamenten bij oudere patiënten (ouder dan 70 jaar) met een kleincellig bronchuscarcinoom. Op korte termijn zal in de zogenaam-

de geindustrialiseerde wereld een steeds groter aantal bejaarden zich presenteren met een kleincellig bronchuscarcinoom alleen al door de demografische ontwikkeling van de laatste jaren. De behandeling met cytostatica heeft vaak veel bijwerkingen, zodat er onder artsen aarzeling bestaat om deze therapie bij oudere patiënten toe te passen. De gegevens uit de literatuur leveren echter geen steun aan een al te defaitistische houding jegens oudere mensen met carcinomen die in aanmerking komen voor palliatieve chemotherapie.

In hoofdstuk 2 worden de resultaten van een fase II onderzoek met oraal toegediend etoposide (totale dosis 800 mg/m^2, verdeeld over 5 achtereenvolgende dagen) bij patiënten ouder dan 70 jaar met een kleincellig bronchuscarcinoom beschreven. Bij 35 niet met chemotherapie voorbehandelde patiënten werd bij 71% een remissie gezien. De mediane overleving was 16 maanden voor patiënten die behoorden tot de zogenaamde "limited disease" groep en 9 maanden voor patiënten met "extensive disease". De bijwerkingen van deze behandeling beperkten zich tot geringe beenmergdepressie en haaruitval. Deze resultaten zijn goed te vergelijken met die welke verkregen worden met combinatie-chemotherapie bij "jongere" patiënten. De behandeling met oraal etoposide vormt een goede palliatieve behandeling voor patiënten ouder dan 70 jaar met een kleincellig bronchuscarcinoom.

Hoofdstuk 3 beschrijft een fase I studie met oraal toegediend teniposide, evenals etoposide een semi-synthetisch podophyllotoxine derivaat. Er zijn aanwijzingen dat dit cytostaticum effectiever is tegen het kleincellig bronchuscarcinoom dan etoposide. Teniposide is nog niet eerder op deze wijze toegediend. De optimale dosering voor fase II onderzoek blijkt 135 mg/m^2 per dag, gedurende 5 achtereenvolgende dagen te zijn. Een belangrijk probleem zijn de bijwerkingen op het maag-darm kanaal (misselijkheid, braken en diarree). Deze bijwerkingen kunnen misschien verminderd worden wannneer capsules teniposide in plaats van een drinkbare oplossing gebruikt worden, zoals ook voor etoposide het geval is. Tot deze capsules ontwikkeld zijn, dient het gebruik van oraal teniposide ontraden te worden.

In hoofdstuk 4 wordt een fase II onderzoek met teniposide 450 mg/m^2 verdeeld over drie dagen (intraveneus) bij patiënten met hersenmetastasen van een kleincellig bronchuscarcinoom beschreven. Bij 11 van de 26 evalueerbare patiënten werd een objectieve remissie in cerebro bereikt, met een mediane duur van 42 weken. De bijwerkingen van deze behandeling lagen vooral op het vlak van de beenmerg depressie, welke echter zelden ernstig was. De beschreven therapie resulteerde in een goede palliatie voor patiënten met hersenmetastasen van een kleincellig bronchuscarcinoom.

In de hoofdstukken 5 en 5^a worden de nefrotoxische eigenschappen van een nieuw platinacomplex, carboplatine, onderzocht. De succesvolle toepassing van cisplatine als antikankergeneesmiddel heeft geleid tot de ontwikkeling van platinacomplexen met als doel een middel te vinden met minder bijwerkingen dan cisplatine, maar met behoud van antitumoraktiviteit. Met name de schadelijke effecten van cisplatine op de nierfunctie hebben daartoe de aanzet gegeven. In een prospectief onderzoek werd de nierfunctie onderzocht bij 10 patiënten met longkanker, behandeld met carboplatine (400 mg/m^2 dag 1) en vincristine (2 mg dag 1 en 8), elke vier weken. Na twee kuren bleek een significante daling

van de glomerulaire filtratie snelheid en de effektieve renale plasma doorstroming te zijn opgetreden. Na vijf kuren bedroeg dit verlies respectievelijk 19% en 14%. In de gelijktijdig gemeten uitscheiding van tubulaire enzymen en relatieve ß$_2$-microglobuline klaring konden geen significante verschillen gemeten worden. De mate van beenmergdepressie veroorzaakt door carboplatine wordt vooral bepaald door de glomerulaire filtratie snelheid, daar dit medicament voor het overgrote deel uit het plasmacompartiment geklaard wordt door de nieren. Bij de patiënten beschreven in dit onderzoek, nam de mate van beenmergdepressie niet gelijktijdig toe met de achteruitgang in glomerulaire filtratie snelheid. Concluderend kan gesteld worden dat ook carboplatine een nefrotoxische stof is.

In de hoofdstukken 6 en 7 worden de effektiviteit en toxiciteit van de combinatie van carboplatine en vincristine bij patiënten met bronchuscarcinoom onderzocht. Bij voorbehandelde patiënten met een kleincellig bronchuscarcinoom werd bij 37% (10 van de 28 patiënten) een partiële remissie gezien (hoofdstuk 6). Bij 22 patiënten die een recidief kregen kort na de inductiebehandeling met andere cytotoxische medicamenten werden 8 partiële remissies gezien. In het algemeen worden dergelijke patiënten op klinische gronden resistent geacht voor de medicamenten gebruikt in de inductiebehandeling. Uit deze waarneming kan geconcludeerd worden dat de gebruikte combinaties tot op zekere hoogte niet kruis-resistent zijn. Het verschil in gevoeligheid tussen kleincellige en niet-kleincellige bronchuscarcinomen voor chemotherapie wordt gedemonstreerd door de resultaten van fase II onderzoek met dezelfde combinatie (carboplatine en vincristine) bij patiënten met een niet-kleincellig bronchuscarcinoom (hoofdstuk 7). Slechts bij 5 van de 30 patiënten kon een kortdurende remissie bereikt worden. Het is nog onduidelijk wat de rol van deze twee medicamenten in de behandeling van het niet-kleincellig bronchuscarcinoom zal zijn.

Hoofdstuk 8 beschrijft een fase I en farmacokinetische studie met 21 dagen continue infusie van carboplatine. Continue infusie is mogelijk een methode om de therapeutische index (de verhouding tussen effektiviteit en toxiciteit) van cytostatica te verhogen. Een dosis van 30 mg/m^2/dag gedurende 21 dagen werd gevonden als optimale dosis voor fase II onderzoek. Patiënten die met deze dosis (in totaal 630 mg/m^2) behandeld werden hadden aanzienlijk minder bijwerkingen vergeleken met gegevens uit de literatuur wanneer dezelfde dosis carboplatine als bolus infusie dtoegediend werd. Farmakokinetische bepalingen werden uitgevoerd met behulp van atomaire absorptie. Steady state spiegels van niet eiwit gebonden platinum (vrij platinum) werden na 8 uur infusie bereikt. Er kon geen verband worden aangetoond tussen de hoogte van de steady state plasma spiegels en de toegediende dosis carboplatine. Voor het eiwit gebonden platinum werd de steady state niet bereikt, er bleek wel een relatie te bestaan tussen toegediende dosis en plasma spiegels. Tevens werden significante relaties gevonden tussen de glomerulaire filtratie snelheid enerzijds en de steady state plasma spiegels van het vrije platinum en de mate van bloedplaatjes depressie anderzijds. De farmokokinetiek van carboplatine wordt dus niet beinvloed door continue infusie. De cytotoxische lesie van platina houdende antikankermiddelen wordt gevormd door zogenaamde platina adducten. Dit zijn platineringsproduk-

ten van het DNA. Bij vijf patiënten werden deze adducten met behulp van een polyclonaal antilichaam gemeten in witte bloedcellen. Er bleek een lineair verband te bestaan tussen de duur van de infusie en de hoeveelheid gevormde adducten in individuele patiënten. Er was echter geen correlatie tussen de expositie van de witte bloedcellen aan vrij platinum en de mate van platinering van het DNA. Deze bevinding dient nog bevestigd te worden in een grotere groep van patiënten. Het geeft wel de relatieve waarde aan van plasma farmacokinetiek van platina bevattende antikankermiddelen.

In hoofdstuk 9 wordt een vergelijkbaar onderzoek beschreven met continue infusie van vindesine. In tegenstelling tot andere vinca-alkaloiden is het voor vindesine niet mogelijk de therapeutische index te verhogen door verlenging van de infusie duur. Op de laagste dosisstap (0.2 mg/m²/dag) was het noodzakelijk de studie te stoppen in verband met ernstige neurotoxiciteit, welke gelukkig reversibel bleek te zijn.

Een andere benaderingswijze van patiënten met bronchuscarcinoom met als doel de bijwerkingen van behandeling te reduceren, is selectie van patiënten met behulp van laboratoriumtechnieken. Indien het mogelijk is op een betrouwbare manier te voorspellen of een individuele patiënt zal reageren op (chemo-)therapie kan onnodige toxiciteit vermeden worden. In hoofdstuk 10 worden 3 kleincellige bronchuscarcinoom cellijnen afkomstig van patiënten die een complete remissie vertoonden na combinatie chemotherapie vergeleken met 3 cellijnen afkomstig van patiënten die geen remissie vertoonden na behandeling met betrekking tot gevoeligheid voor cytostatica *in vitro*. Voor dit doel werd de micro titerwell assay gebruikt. Een correlatie tussen de *in vivo* en *in vitro* respons kon niet gevonden worden. Om een verklaring voor dit feit te vinden werden 5 van de 6 cellijnen vergeleken met de tumor biopten van waaruit zij verkregen werden. Door kleuring met een aantal monoclonale antilichamen konden slechts kleine verschillen aangetoond worden. Twee cellijnen konden met de oorspronkelijke tumor biopten vergeleken worden met betrekking tot DNA ploidie. Op basis van verschillen in DNA ploidie werd geconcludeerd dat er selectie *in vitro* optreed van tumorcelpopulaties. Dit lijkt de meest aannemelijke verklaring te zijn waarom er geen correlatie was met de *in vivo* response. Kleincellige bronchuscarcinoom cellijnen zijn dus geen geschikt model voor selectie van patiënten voor aanvang van de behandeling zoals boven bedoeld. Dit neemt niet weg dat cellijnen een onmisbaar instrument zijn om de verschillende vormen van resistentie tegen cytostatica in het laboratorium te bestuderen.

In hoofdstuk 11 staan de resultaten beschreven van een prospectief onderzoek van *in vitro* predictive testing op vers humaan tumor materiaal onder andere afkomstig van patiënten beschreven in de hoofdstukken 6 en 7. In dit onderzoek werd de fast green assay gebruikt. Bij de in totaal 66 verkregen tumorbiopten was het in 23 gevallen mogelijk een uitspraak te doen over de *in vitro* gevoeligheid voor de gebruikte cytostatica. De voornaamste reden voor het mislukken van de assay was gelegen in de onvoldoende opbrengst van tumorcellen met behulp van bronchoscopie en onvoldoende overleving van de tumorcellen gedurende 4 dagen *in vitro* in de controles. De bruikbaarheid voor *in vitro* predictive testing van de fast green assay bij bronchuscarcinomen is dus gering.

Concluderend kan gesteld worden dat de resultaten van palliatieve behandeling van patiënten met longkanker op verschillende wijzen verbeterd kunnen worden. Patiënten ouder dan 70 jaar die zich presenteren met een kleincellig bronchuscarcinoom kunnen behandeld worden met een eenvoudige en relatief niet toxische vorm van chemotherapie. Patiënten met hersenmetastasen van een kleincellig bronchuscarcinoom kunnen behandeld worden met chemotherapie alleen. Prospectief klinisch onderzoek is nodig om te kunnen beslissen over de waarde van radiotherapie alleen of toegevoegd aan chemotherapie in deze patiëntengroep. Carboplatine is een werkzaam cytostaticum bij het kleincellig bronchuscarcinoom. In het bijzonder dient van dit medicament de mogelijke niet kruisresistente eigenschap in de kliniek ten opzichte van veelgebruikte cytostatica bij het kleincellig bronchuscarcinoom onderzocht te worden. Continue in plaats van bolus infusie carboplatine heeft minder bijwerkingen op het beenmerg en de nierfunctie. Deze voordelen, en vergelijking van de antikankeraktiviteit van deze wijzen van toediening, moeten in gerandomiseerde studies onderzocht worden.

Voor verdere verbetering van het lot van patiënten met longkanker zijn nieuwe behandelings strategieën nodig. Van groot belang zal het onderzoek (*in vitro* en *in vivo*) naar de verschillende vormen van resistentie tegen cytostatica blijken te zijn. Nieuwe diagnostische middelen zoals die aangereikt worden vanuit de immunologie en moleculaire biologie maken het mogelijk om deze mechanismen in humaan tumor materiaal te bestuderen op cellulair nivo. Tevens dienen er technieken ontwikkeld te worden om te kunnen voorspellen of de veronderstelde resistentie mechanismen aanwezig en van functionele betekenis zijn *in vivo*. Nieuwe wijzen van behandeling gestoeld op de resultaten van bovengenoemde technieken dienen ontwikkeld te worden. Alleen dan zal voor patiënten met een inoperabel bronchuscarcinoom zowel de verbetering van de kwaliteit van het leven als belangrijke verlenging of zelfs genezing het doel kunnen zijn van cytostatische therapie.