dopamine might be inefficient and might cause additional damage to tubules and glomeruli and affirm that only potent vasoconstrictor agents might be useful in this clinical situation [22]. These observations contrast with our results: we have in fact demonstrated significant improvement of renal function during "low dose" dopamine infusion in acute renal failure induced by rIL-2.

In conclusion this is the first report which shows that the use of "low dose" dopamine by continuous infusion controls prerenal azotaemia, serum creatinine levels and oliguria in patients who are at high risk and finally develop major rIL-2 induced renal toxic effects and determines an early recovery of the rIL-2—impaired renal function. Administration of low-dose dopamine, therefore, can avoid dose reduction or discontinuation of rIL-2 administration, which could compromise the antitumour effect of rIL-2.

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Eur J Cancer, Vol. 29A, No. 8, pp. 1122–1127, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Phase I Study of WR-2721 and Carboplatin

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Because WR-2721 reduces the toxicity of cisplatin and carboplatin in preclinical systems, we have treated 35 patients in a phase I study of WR-2721 and carboplatin. As the plasma half-life of WR-2721 is short relative to that of carboplatin, WR-2721 was administered in two divided doses. This schedule produced acceptable toxicity in 24 patients treated with carboplatin 400 mg/m² and escalating doses of WR-2721. In the subsequent 11 patients, WR-2721 was fixed at 740 mg/m²/dose and the dose of carboplatin was escalated. With WR-2721, grade 3–4 thrombopenia (platelets $<50 \times 10^9$/l) was produced in 4/5 patients treated with carboplatin 625 mg/m² and in 1/6 patients treated with carboplatin 500 mg/m². Carboplatin pharmacokinetic parameters in 4 patients were similar to those reported for carboplatin alone. These results suggest that WR-2721 might increase the maximum tolerated dose of carboplatin from 400 to 500 mg/m².
Eur J Cancer, Vol. 29A, No. 8, pp. 1122–1127, 1993.

INTRODUCTION

WR-2721 [S-2-(3-aminopropylamino) ethyl phosphorothioic acid, Ethiofos] is a sulfhydryl compound which selectively protects normal tissues from the cytotoxicity of radiation and alkylating agents in animal models [1]. The mechanism by which this selective protection occurs requires further study, but has been attributed to a differential metabolism of the agent by normal and neoplastic tissues [2]. WR-2721 is rapidly converted to the active species, WR-1065 and related disulphides, which are rapidly taken up into normal tissues [3, 4]. *In vitro* studies

suggest that alkaline phosphatase may be important in catalysing the conversion of WR-2721 to WR-1065, and further demonstrate that intracellular concentrations of WR-1065 correlate with radioprotection [4, 5]. Differences in alkaline phosphatase activity between normal and malignant tissues may account for the differential absorption of the active species [4, 5].

In BALB/c mice and Fisher 344 rats, WR-2721 attenuated the myelosuppression produced by cyclophosphamide and cisplatin [6]. There was no evidence of protection of the tumour by WR-2721 from the cytotoxicity of the chemotherapeutic agents [6]. Preclinical trials of WR-2721 and carboplatin have been performed as well. *In vitro* studies have shown that WR-2721 and its active metabolite WR-1065 inhibit the platination of DNA and, to some extent, reverse platinum complex/DNA adducts [7]. WR-2721 400 mg/kg administered intraperitoneally reduced the degree of leukopenia produced by the intraperitoneal administration of carboplatin 100 mg/kg in $CD2F_1$ mice from 49% of control to 74% of control [8]. Pretreatment with WR-2721 reduced the myelosuppression produced by carboplatin in mice, with a suggestion of increased antitumour activity of the combination against OVCAR-3 xenografts compared with that of carboplatin alone being observed [9].

Clinical trials have investigated the effects of WR-2721 on the toxicity of cisplatin [10, 11], hemibody radiation [12] and cyclophosphamide [13, 14]. Treatment with WR-2721 600–900 mg/m^2 prior to hemibody radiation was reported to produce less myelosuppression than treatment with hemibody radiation alone in a study with a non-randomised control group [12]. While an uncontrolled study of WR-2721 and cyclophosphamide did not demonstrate that WR-2721 could affect the myelosuppression of cyclophosphamide 1000 mg/m^2 [13], a controlled study did show that the myelosuppression produced by cyclophosphamide could be attenuated by cotreatment with WR-2721 [4]. In a study of WR-2721 with escalating doses of cisplatin, minimal nephrotoxicity was reported with doses of cisplatin of up to 150 mg/m^2, but further dose escalation was not possible due to neurotoxicity attributed to cisplatin [10]. While the nephrotoxicity of the combination of WR-2721 and cisplatin was suggested to be less than expected for cisplatin alone at the doses given, the maximum tolerated dose (MTD) of cisplatin when given with WR-2721 was not significantly greater than that of cisplatin alone.

Carboplatin may be a better drug to use in conjunction with WR-2721 than cisplatin, as it is less neurotoxic than the parent drug, and it is now clear that neurotoxicity is the dose limiting toxicity of cisplatin as a single agent with or without WR-2721 [10, 11, 15]. Rat and murine bone marrow cells avidly absorb WR-2721, leading to radio- and chemoprotection [1–5, 16]. Because myelosuppression is the dose-limiting toxicity of carboplatin, clinical studies of this drug in combination with WR-2721 would be of interest. Based upon the rationale constituted by the preclinical and clinical studies discussed above, we have performed a phase I trial of the combination of WR-2721 and carboplatin.

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Revised 26 Oct. 1992; accepted 20 Nov. 1992.

PATIENTS AND METHODS

Study design

WR-2721 was administered prior to carboplatin and again 2 h after carboplatin. This schedule was based upon pharmacokinetic differences between carboplatin and cisplatin. Carboplatin has a more delayed excretion, the beta half-life being 2–4 h [17–20]. Because WR-2721 has a very short plasma half-life [21, 22], greater myeloprotection might be afforded by multiple than by single doses when this agent is given with drugs that have relatively long plasma half-lives, such as carboplatin. Our phase I trial of WR-2721 and carboplatin was performed in two phases. In the first phase (A), a phase I trial of WR-2721 with a fixed dose of carboplatin was performed in order to determine whether two doses of WR-2721 could be administered in a single day. In this phase of the study, it was demonstrated that two doses of WR-2721 740 mg/m^2 could be administered with acceptable toxicity. In the second phase (B) of the study, the dose of WR-2721 was fixed at 740 mg/m^2/dose and administered once prior to carboplatin and again 2 h after carboplatin. The dose of carboplatin was escalated in successive cohorts of 4–6 patients.

Patient selection

This trial was open to patients with measurable or evaluable advanced malignancies refractory to standard therapy or for which no effective standard therapy was felt to exist. Patients were required to be of SWOG performance status 0–2, age 18 or greater, and to have adequate major organ function, defined as white blood count $\geq 3.5 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, haemoglobin ≥ 9.0 g/dl, total bilirubin ≤ 1.5 mg/dl and serum creatinine ≤ 1.5 mg/dl or creatinine clearance ≥ 60 ml/min. At least 3 weeks were required to have passed since any surgery, chemotherapy or radiation therapy. The study was approved by the Institutional Review Board and patients were required to give their informed consent to participate. Patients were excluded from participation if they had New York Heart Association Class 3–4 angina, were pregnant, or were not practising contraception. The sum of prior chemotherapeutic regimens for metastatic or recurrent disease plus the number of courses of radiotherapy of ≥ 30 Gy applied to $\geq 1/3$ of the axial skeleton was required to be ≤ 2 during phase A of the study and ≤ 1 during phase B of the study. After excessive myelosuppression was noted in patients who had received prior mitomycin-C, patients who had received prior nitrosoureas or intravenous mitomycin-C were excluded. Prior biological response modifier therapy was not restricted. No concurrent radiotherapy or chemotherapy was allowed.

Drug administration and evaluation

WR-2721 was reconstituted in sterile water and administered in normal saline. WR-2721 was administered as a 15-min infusion once 15 min prior to the administration of carboplatin and again 2 h after the administration of carboplatin. Intravenous fluids were given beginning 15 min prior to the first dose of WR-2721 and continued until the completion of the last dose of WR-2721. Blood pressure determinations were made every 2 min during WR-2721 administration, and treatment was temporarily held in the event that the systolic blood pressure fell by ≥ 25 mmHg. Treatment was restarted when the blood pressure had recovered to normal. Carboplatin was administered over 5–10 min, beginning 15 min after the first dose of WR-2721. Most patients were treated as outpatients every 4 weeks. Some patients treated on the lower dose levels were treated every 3 weeks. Haematological, biochemical and clinical recovery from

the toxicity of the previous course of therapy were required before retreatment. Treatment was discontinued in non-responding patients after two cycles of therapy; responding patients were treated until they experienced disease progression or unacceptable toxicity.

Dose escalation

The first dose of WR-2721 was 740 mg/m^2 in all patients. During phase A of the study, the dose of carboplatin was fixed at 400 mg/m^2 and the second dose of WR-2721 was escalated to 185, 370, 555 and 740 mg/m^2. Acceptable toxicity was observed at all of these dose levels, so that during phase B of the study, all patients received two doses of WR-2721 of 740 mg/m^2 each. In phase B, the dose of carboplatin was escalated to 500 and 625 mg/m^2. Unacceptable toxicity was produced by a dose of carboplatin of 625 mg/m^2, so that no further dose escalations were made.

Carboplatin pharmacokinetics

Because one mechanism by which WR-2721 might affect the toxicity of carboplatin would be through an alteration in the pharmacokinetics of carboplatin, we studied the pharmacokinetics of carboplatin in 4 patients entered on phase B of the study. Plasma samples were drawn once prior to drug administration and again 15, 30, 60, 90, 120, 240, 300 and 1440 min after carboplatin administration.

Blood samples were collected in prechilled (4°C) heparinised tubes and centrifuged for 20 min at 700 *g* to obtain plasma. Ultrafiltrate was subsequently obtained by centrifuging the plasma at −4°C through Amicon Centriflo CF-25 cones (25 000 MW cut-off) at 1000 *g* for 30 min. The ultrafiltrate was stored frozen at −20°C prior to analysis of platinum levels by flameless atomic absorption spectrophotometry as described earlier by Priesner [23] and Andrews [24].

Pharmacokinetic analysis of plasma platinum levels were carried out by using a curve stripping program for parameter estimates [25] coupled with a non-linear regression analysis program (PCNONLIN^R [26]) for fitting plasma platinum level data using a two-compartment kinetic model with constant intravenous input and first order output.

Table 1. Clinical characteristics

Number registered	35
Number eligible	35
Number evaluable	35
Male:female	22:13
Age (years)	
Median	61
Range	21–73
ECOG performance status	
0	4
1	22
2	9
Primary diagnoses	
Colon cancer	8
Lung cancer	6
Breast cancer	4
Bladder cancer	3
Pancreatic cancer	2
Biliary cancer	2
Melanoma	2
Sarcoma	2
Other	6
Prior therapy (may be > 1 per patient)	
Chemotherapy	22
Radiation	13
Biological therapy	9
Hormonal therapy	4
None	5
Serum creatinine (mg/dl)	
Mean	1.0
Range	0.6–1.6
Calculated creatinine clearance (ml/min)	
Mean	85
Range	40–143

RESULTS

Patient population

A total of 35 patients were registered on this study; 24 were treated on the WR-2721 dose-ranging phase of the study (A) and 11 were treated on the carboplatin dose-escalation phase of the study (B). All registered patients were eligible and evaluable. The characteristics of the study population are summarised in Table 1.

Toxicity

All toxicities were graded according to the common toxicity criteria of the National Cancer Institute. The toxicities observed at each dose level are summarised in Tables 2 and 3. The median number of courses was two; similar conclusions are reached if only the toxicities observed after the first course of therapy are analysed, as doses were modified according to toxicity. Grade 3–4 lymphopenia was observed at all dose levels. Because this toxicity was associated with no symptoms or identifiable adverse consequences, dose escalation continued despite grade 3–4 lymphopenia. Grade 2 nausea and vomiting was produced in most patients. Hypotension was noted during WR-2721 infusion, but was rapidly reversible in all cases by temporarily discontinuing drug administration. No patient required therapy with vasopres-

Table 2. Phase I trial of WR-2721 + CBDCA

	Grade	Non-haematological toxicity by dose level					
Dose CBDCA		400	400	400	400	500	625
WR-2721 pre		740	740	740	740	740	740
WR-2721 post		185	370	555	740	740	740
Patient no.		6	7	5	6	6	5
Toxicity							
Alkaline phosphatase	1	3	1	2	1	4	2
	2	1	1	0	1	0	1
	3	0	2	1	2	0	0
	4	0	1	1	0	0	0
Hypocalcaemia	1	1	2	1	1	1	0
	2	2	0	0	1	2	1
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
Hypotension	1	3	3	3	5	3	4
	2	0	1	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
N/V	1	0	1	0	0	0	1
	2	6	5	5	6	4	3
	3	0	0	0	0	1	0
	4	0	0	0	0	0	0

Table 3. Phase I trial of WR-2721 + CBDCA

		Grade Haematological toxicity by dose level					
Dose CBDCA		400	400	400	400	500	625
WR-2721 pre		740	740	740	740	740	740
WR-2721 post		185	370	555	740	740	740
Mean pretreatment							
Serum Cr (mg/dl)		1.13	0.99	0.94	0.88	0.95	1.08
Calculated ClCr							
(ml/min)		72.3	92.2	76.5	90.6	92.7	82.6
Patient no.		6	7	5	6	6	5
Toxicity							
Leucopenia	1	0	1	0	0	0	0
	2	0	1	2	0	2	1
	3	3	3	1	2	2	3
	4	2	1	2	4	2	1
Neutropenia	1	1	1	1	0	0	1
	2	2	0	0	2	1	0
	3	0	1	1	2	0	1
	4	0	0	0	0	1	2
Thrombocytopenia	1	3	2	4	1	2	0
	2	0	1	0	1	0	1
	3	2	0	0	2	0	0
	4	1	1	1	1	1	4

Cr: Creatinine; ClCr: creatinine clearance.

sors. The abnormalities of the alkaline phosphatase were attributed to disease progression rather than drug toxicity. At dose level A4 (WR-2721 740 mg/m^2 before and after carboplatin 400 mg/m^2), grade 3–4 thrombopenia was noted in 3 of 6 patients. 2 patients at this dose level had been previously treated with mitomycin-C. Because it was felt that this prior therapy was more likely to have accounted for the toxicity than was the dose of escalation of WR-2721, the protocol was amended to exclude patients who had received nitrosoureas or intravenous mitomycin-C as well as patients who had received two or more myelosuppressive therapies for metastatic disease, and phase B was initiated. Treatment during phase B of the study was, in general, administered as scheduled. During phase B, the median interval between treatments was 28 days; the mean treatment interval was 31 days (range 28–44), with 2 patients having courses delayed.

Unacceptable toxicity was observed at a dose of carboplatin of 625 mg/m^2, administered with two doses of WR-2721 of 740 mg/m^2 each. At that dose level, 4 of 5 patients experienced grade 4 thrombopenia and 3 experienced grade 3–4 neutropenia. The next lower dose of carboplatin, 500 mg/m^2, was the MTD of carboplatin that could be given with two doses of WR-2721 of 740 mg/m^2 each. At that dose level, 1 patient experienced grade 4 thrombocytopenia and grade 4 neutropenia.

We attempted to predict the degree of thrombocytopenia for each patient, using the model described by Egorin *et al.* [27]. A modification of the formula was used in heavily pretreated patients (total number of courses of radiation therapy + chemotherapy $\geq$ 2). In these formulae, the creatinine clearance is one of the major determinants of the change in platelet count. We estimated our patients' creatinine clearances, based upon the serum creatinine, age and weight, by applying a widely used formula [28]. Only 3 of our patients had been previously treated with cisplatin, and none had received this agent within 8 weeks of treatment on our study, so any effect of prior cisplatin therapy on the assessment of renal function in our patient population is likely to have been minimal. As demonstrated in Fig. 1, the percentage change in platelet count was related to that predicted by the mathematical models derived from patients receiving carboplatin without WR-2721 [27] (Spearman correlation coefficient, $\rho = 0.39$, $P = 0.02$), though this relationship was not as close as previously reported.

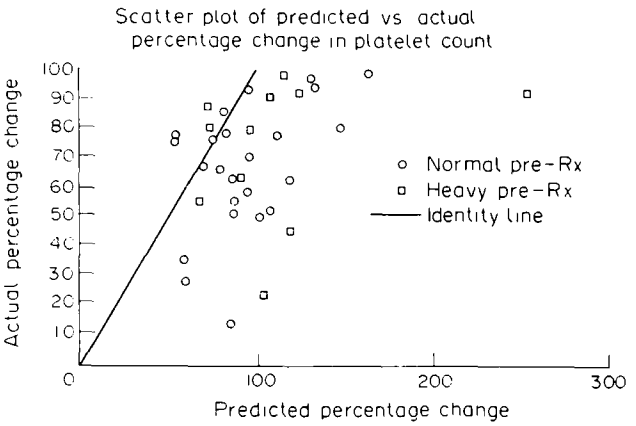

Fig. 1. Predicted vs. observed changes in platelet counts.

Efficacy

A single patient with non-small cell lung cancer, treated at dose level 3A (carboplatin 400 mg/m^2, first dose of WR-2721 740 mg/m^2, second dose of WR-2721 555 mg/m^2), developed a partial response to therapy which persisted for 27 weeks. This relative lack of demonstrable antitumour activity is not unexpected in this patient population.

Carboplatin pharmacokinetics

The results of the pharmacokinetic studies are summarised in Table 4. The median alpha half-life was 11 min and the median beta half-life was 145 min, using a two compartment model. For each of these 4 patients, the area under the concentration/time curve (AUC), calculated creatinine clearance [28], and observed decrease in platelet count following the first course of therapy are also shown. Greater degrees of change in the platelet count were associated with higher AUC, consistent with previously reported studies involving larger numbers of patients treated with carboplatin without WR-2721 [18, 27].

DISCUSSION

WR-2721 can be given twice daily in doses of 740 mg/m^2 each with acceptable toxicity. The dose of WR-2721 could likely be

Table 4. Pharmacokinetic parameters for ultrafilterable platinum and comparison with calculated creatinine clearance and % change in first course platelet count

Dose (mg/m^2)	Calculated ClCr (ml/min)	Alpha half-life (min)	Beta half-life (min)	C_{max} (ng/ml)	AUC_{0-24} (μg/ml hr elemental Pt)	% drop platelets
500	87	7	145	77.7	81	58
500	104	27	379	22.1	39	55
625	104	13	106	49.5	78	62
625	94	9	144	27.2	97	97

ClCr: Creatinine clearance; AUC: area under curve.

escalated further, as no unacceptable toxicity attributable to WR-2721 was encountered during the WR-2721 dose escalation-phase of our study. Further dose escalations were not explored because a dose of WR-2721 of 740 mg/m^2 had been recommended for use with cisplatin in a previous study [10]. A divided dose schedule was studied because the plasma half-life of WR-2721 is short relative to that of carboplatin; more than 90% of WR-2721 was found to be cleared from plasma within 6 min of the completion of a 15-min infusion of WR-2721 in one study [22]. Murine studies have shown that the active metabolite of WR-2721, WR-1065, is detectable in normal tissues after WR-2721 has disappeared from plasma [3, 22]. However, WR-1065 was detectable in normal tissues at a relatively low concentration 30–180 min after drug administration in these animal studies [3, 22]. These data support the rationale for multiple-dose or infusion schedules of administration of WR-2721 when this protectant is used with agents whose parent drugs or active metabolites have relatively long half-lives, such as carboplatin, cyclophosphamide or ifosfamide.

Previous studies of carboplatin alone have demonstrated an alpha half-life of 6–25 min and a beta half-life of 100–250 min [17–20]. These results are similar to the pharmacokinetic parameters derived from our patients. This suggests that WR-2721 does not significantly affect the pharmacokinetics of carboplatin.

The MTD of carboplatin that could be given in conjunction with two doses of WR-2721 was 500 mg/m^2. This should be compared with the MTD reported in phase I studies of carboplatin alone. Most phase I studies of carboplatin alone have suggested a dose of 400–500 mg/m^2 for phase II studies, with the higher dose being recommended for previously untreated patients or for patients receiving carboplatin in divided doses over 5 days [17, 20, 29, 30]. One trial identified a dose of 320 mg/m^2 as the MTD for carboplatin administered as a 24-h infusion, but this may have been related to the prior treatment of patients entered on this study [19]. Our patient population consisted of patients who were allowed to have been treated previously, but not extensively. The prior therapy status of our patients does not seem to have been an important determinant of the MTD identified in our study, as none of the 5 patients treated at the highest dose of carboplatin (625 mg/m^2) had received prior chemotherapy, while 3 of the 6 patients treated at the next lower dose (MTD, 500 mg/m^2) had received prior cytotoxic therapy. Examination of Fig. 1 suggests that, for most patients in our study, the observed change in the platelet count was less than that predicted by the models developed by Egorin *et al.* [27]. This might reflect an effect of WR-2721, but might also be due to the use of an estimate of glomerular filtration rate or to differences between our patient population and that from which the formulas were derived.

We conclude, then, that (1) two doses of WR-2721 of 740 mg/m^2 each can be safely administered, and (2) the MTD of carboplatin when given with two doses of WR-2721 is 500 mg/m^2. Considering our patient population and the previously reported experience with carboplatin, these results suggest the hypothesis that WR-2721 attenuates the toxicity of carboplatin, raising the MTD of carboplatin from 400 to 500 mg/m^2. This hypothesis is currently being tested in a randomised prospective trial.

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Eur J Cancer, Vol. 29A, No. 8, pp. 1127–1132, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

In vivo Biological Results of the Association Between Interleukin-2 and Interleukin-3 in the Immunotherapy of Cancer

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The concomitant generation of macrophage-mediated suppressive events, as documented by the increase in neopterin and soluble interleukin-2 (IL-2) receptor (SIL-2R), and the enhanced production of cortisol, would represent the most investigated phenomena responsible for the reduced anticancer efficacy of IL-2 immunotherapy in humans. Based on our preliminary experimental studies suggesting a modulatory role of IL-3 on immune and endocrine effects induced by IL-2, a study was performed to evaluate the influence of IL-3 on biological effects of IL-2 cancer immunotherapy. We have evaluated 12 immunotherapeutic courses with IL-3 plus IL-2, which were performed in 6 patients with metastatic non-small cell lung cancer. The results were compared to those seen in 22 courses with IL-2 alone, carried out in 12 patients with metastatic non-small cell lung cancer. IL-3 was given intravenously at a daily dose of 1 μg/kg/b.w. at 6 p.m. for 14 consecutive days, starting 7 days before IL-2. IL-2 was given subcutaneously at a dose of 3 million IU twice/daily for 5 days/week for 3 weeks. The increase in serum levels of the specific macrophage marker neopterin, induced by IL-2, was completely blocked by IL-3. The IL-2-induced SIL-2R rise was significantly lower during IL-3 plus IL-2 than under IL-2 alone. The increase in cortisol levels in response to IL-2 was neutralised by IL-3. The increase in lymphocyte, T lymphocyte, natural killer (NK) cell, activated T lymphocyte and eosinophil mean number was significantly higher during IL-3 plus IL-2 than during IL-2 alone. Episodes of fever, asthenia, anorexia, vomiting, anaemia and thrombocytopenia were significantly more frequent in patients receiving IL-2 alone than in those treated with IL-3 and IL-2. This preliminary study would suggest that IL-3 may improve the tolerability of IL-2 immunotherapy and enhance the biological antitumour properties of IL-2 by neutralising cortisol increase and macrophage-mediated suppressive events, with a following potential amplification of Il-2 anticancer efficacy.
Eur J Cancer, Vol. 29A, No. 8, pp. 1127–1132, 1993.

INTRODUCTION

IT IS known that lymphokine-activated killer (LAK) cells, generated by interleukin-2 (IL-2) from natural killer (NK) cells and T lymphocyte precursors may destroy *in vitro* almost all tumour histotypes [1]. On the other hand, only few tumour histotypes seem to respond *in vivo* to IL-2, mainly renal adenocarcinoma and malignant melanoma [2–4]. It has to be considered that IL-2 administration induces not only immune effects, but also metabolic [5, 6], endocrine [7], neuroendocrine [8, 9] and cardiovascular effects [10]. The causes responsible for the low *in vivo* anticancer efficacy of IL-2 could be related to a greaty variety of immunosuppressive events [11, 12], mediated either by the endocrine system or by the immune system. Furthermore, the following suppressive events have been described during IL-2 cancer immunotherapy: (1) stimulation of cortisol secretion [7–9], which plays an inhibitory effect on IL-2-dependent immune functions [13]; (2) abnormal increase in soluble IL-2 receptors (SIL-2R) [12,14], which bind IL-2 by

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Revised 3 Sep. 1992; accpeted 19 Oct. 1992.