Pharmacokinetics of recombinant human interleukin-2 in advanced renal cell carcinoma patients following subcutaneous application

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Aims The aim of the study was to investigate the pharmacokinetics of recombinant human interleukin-2 (rhIL-2) in patients with metastatic renal cell carcinoma following different subcutaneous (s.c.) administration regimens.

Methods RhIL-2 was administered subcutaneously to 10 patients according to two different dosing regimens: group A received 20×10^6 IU m^{-2} once daily and group B 10×10^6 IU m^{-2} twice daily (every 12 h). Additionally, in all patients the influence of soluble interleukin-2 receptor (sIL-2R) on the pharmacokinetics of rhIL-2 was investigated.

Results The mean area under the serum concentration-time curve to 24 h (AUC(0,24 h)) was 627 IU ml^{-1} h in treatment group A and 1130 IU ml^{-1} h ($P=0.029$) in treatment group B. In both study groups C_{max} and AUC(0,12 h) were not significantly different. Seventy-two hours after the beginning of s.c. rhIL-2 therapy the sIL-2R increased significantly ($P=0.016$), and sIL-2R levels over 1200 pmol l^{-1} seemed to reduce the AUC.

Conclusions In patients with metastatic renal cell cancer administration of 20×10^6 IU m^{-2} of rhIL-2 s.c. in two daily doses (10×10^6 IU m^{-2} every 12 h) provides better bioavailability and is preferable to the single dose administration.

Keywords: interleukin-2, pharmacokinetics, soluble interleukin-2 receptor, subcutaneous

Introduction

The use of recombinant human interleukin-2 (rhIL-2) has been recommended as the best current therapy for advanced renal cell carcinoma [1, 2]. RhIL-2 was found to exert its antitumour activity via indirect effects on the immune system, including the activation and expansion of cytotoxic T-lymphocytes and natural killer cells, and the secretion of secondary cytokines such as interferon-γ and TNF-α [3, 4]. The immune modulatory capacity has been described for the i.v. administration of high dose rhIL-2, which was associated with severe adverse effects, including capillary leak-related weight gain, hypotension, malaise, fever, and chills. Subcutaneous (s.c.) rhIL-2 at doses far below the maximum tolerable dose is therapeutically effective while treatment-related toxicity is reduced [5–7]. The pharmacokinetics of intravenously and intramuscularly administered rhIL-2 are well known [8–10], but only limited data on the pharmacokinetics after subcutaneous administration are available and information on the comparison of various dose regimens of s.c. rhIL-2 is lacking.

During systemic administration of rhIL-2 in humans, elevated soluble IL-2 receptor levels have been found [11]. The soluble IL-2 receptor (sIL-2R) differs from its membrane-bound counterpart with respect to size, binding capacity and ligand specificity. The soluble form of the human IL-2 receptor is a glycosylated protein with a molecular weight between 35 and 50 kDa [12]. It binds to IL-2 with low affinity (K_d: 10 nmol l^{-1}) which is comparable with the affinity to the membrane bound α-chain of the IL-2 receptor [13]. The α-chain is expressed on activated T cells which combines with the β-and γ-chains to constitute a high-affinity IL-2R with a 1000-fold higher affinity to IL-2 than presented by the sIL-2R [14–17]. It has been hypothesized that the competition between the membrane-bound and the soluble receptor for IL-2 causes inhibition of IL-2 dependent mechanisms. However, so far, no data are available on the influence of sIL-2R on IL-2 pharmacokinetics.

The present study attempts to provide a more detailed analysis of the pharmacokinetics of subcutaneous rhIL-2 in patients with metastatic renal cell carcinoma comparing different dosing regimens. In addition, pharmacokinetic data of s.c. rhIL-2 were analysed in relation to different concentrations of the sIL-2 receptors.

Methods

Patients and treatment

The study cohort consisted of 10 patients (mean age 56 ± 9 years) treated at our institution with histologically confirmed metastatic renal cell cancer in a clinically progressive stage; all patients had a Karnofsky performance status $>70\%$. No chemotherapy or immune modulatory therapy was per-

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formed for at least 4 weeks prior to this protocol. After obtaining written informed consent patients were treated with s.c. rhIL-2 (Chiron, Emeryville, USA). Two different priming doses of rhIL-2 (1 IU = 2.916 pg) were administered in the first week of therapy on 3 consecutive days: seven patients (mean age 58 ± 8 years; mean body weight 71 ± 11 kg) received 20×10^6 IU m^{-2} rhIL-2 s.c. once daily (group A) and three patients (mean age 50 ± 10 years; mean body weight 69 ± 12 kg) received 10×10^6 IU m^{-2} rhIL-2 s.c. twice daily at a 12 h interval (group B). This study was approved by the institutional ethical committee of the Medizinische Hochschule Hannover.

Sampling

Patients' sera were obtained and stored at −20° C until analysis. The samples for rhIL-2 determination were collected immediately before (0 time) and 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 14, 16, 20, 22 and 24 h after rhIL-2 s.c. injection. Additional sera were prepared for the determination of the soluble interleukin-2 receptor concentration prior to rhIL-2 injection and at least every 4 h during 24 h in all 10 patients. Concentrations of rhIL-2 and sIL-2R were determined in two patients of each treatment group during 3 days of treatment and in one patient of each treatment group during 2 days of treatment.

Interleukin-2 assay

To assay the serum concentration of rhIL-2 a commercial standard cytokine ELISA kit (Medgenix, Ratingen, Germany) was used and performed according to the manufacturer's guidelines. Standard samples, which were contained in the ELISA kit, were used. The lower limit of quantification was 0.5 IU ml^{-1}. A three-run validation was performed to verify the precision and accuracy of the assay. The precision was expressed as the coefficient of variation (CV) of the measured concentrations. The within-run precision ($n = 5$) for the assay at nominal concentrations of 10, 35 and 70 IU ml^{-1} was 3.6%, 5.7%, and 3.9%, respectively. The between-run precision of the assay at these concentrations was 6.1%, 7.5%, and 7.7%, respectively.

ELISA for soluble IL -2R (sIL -2R)

Soluble IL-2R levels were determined using a standard two-step sandwich assay (Immunotech, Marseille, France), as described previously in detail [18]. The amount of sIL-2R per sample was calculated by plotting the absorbance values against a sIL-2R standard curve. Normal donor values ranged from 25 to 115 pmol l^{-1} (1 pmol l^{-1} = 42 pg ml^{-1}). The lower limit of quantification was 5 pmol l^{-1}. The within-run precision was assessed by performing an analysis at three defined concentrations (25, 400, and 800 pmol l^{-1}; $n = 5$). The coefficient of variation was 6.6%, 4.9%, and 5.3%, respectively. The between-run precision of the assay at the above mentioned concentrations was 11.4%, 7.9%, and 9.5%, respectively.

Pharmacokinetic analysis

Compartment independent pharmacokinetic parameters of rhIL-2 were evaluated using the TopFit version 2.0 software [19]. The maximal concentration (C_{max}), the corresponding time (t_{max}) and area under the curve (AUC(0,24 h)) were determined. The elimination half-time ($t_{1/2,z}$) was calculated from the log-linear terminal slope (4–24 h in patients who received 20×10^6 IU m^{-2}; and 4–12 h in patients who received 10×10^6 IU m^{-2} twice daily). For statistical analysis the Wilcoxon's rank sum test was used to compare both treatment regimens. Data are given as means ± s.d.

Results

Subcutaneous administration of 20×10^6 IU m^{-2} rhIL -2 once daily

Seven patients of study group A were treated with s.c. administration of 20×10^6 IU m^{-2} once daily. The pharmacokinetic profile of the mean serum rhIL-2 concentration is presented in Figure 1(a). The pharmacokinetic parameters including t_{max}, C_{max}, $t_{1/2,z}$ and AUC are summarized in Table 1. In group A the mean AUC(0,24 h)-value was 627 + 153 IU ml^{-1} h and the apparent harmonic mean $t_{1/2}$-value 5.1 + 1.1 h. Mean C_{max} was 72 + 20 IU ml^{-1} which was reached at a time of 4.0 + 1.2 h. The mean AUC value (AUC(0,12 h)) was 501 + 125 IU h ml^{-1} (Table 1).

Subcutaneous administration of 10×10^6 IU m^{-2} rhIL -2 every 12 h (twice daily)

Patients of the treatment group B received 10×10^6 IU m^{-2} of rhIL-2 s.c. every 12 h (three patients). In group B C_{max} of rhIL-2 in the first 12 h period amounted to 89 ± 25 IU ml^{-1} at a t_{max} of 4.0 ± 0 h. During the second 12-h period after the administration of 10×10^6 IU m^{-2} rhIL-2 the mean C_{max} was 82 ± 31 IU ml^{-1} at a t_{max} of 2.7 ± 1.7 h (shown in Table 2). The mean AUC(0,12 h) of the first 12 h was 576 ± 126 IU ml^{-1} h, that of the second 12 h period (AUC(12,24 h)) was 554 ± 184 IU ml^{-1} h. Both mean AUC values (AUC(0,24 h)) added up to 1130 IU ml^{-1} h. The pharmacokinetic profile of the mean rhIL-2 concentrations is shown in Figure 1b.

Comparison of the pharmacokinetic data of both study groups

We investigated the influence of the dosage of rhIL-2 on the C_{max}, t_{max} and AUC values after administration of single dose of 10×10^6 IU m^{-2} or 20×10^6 IU m^{-2} rhIL-2 s.c. 4.0 h after rhIL-2 administration t_{max} was reached in both study groups. The mean C_{max} values of group A and group B (72 IU ml^{-1} *vs* 89 IU ml^{-1}) were not significantly ($P = 0.209$) different. We also compared the mean AUC(0,12 h) values of both study groups (501 IU ml^{-1} h *vs* 576 IU ml^{-1} h) and found no significant difference ($P = 0.305$). On the contrary, the AUC(0,24 h) following a twice daily administration of 10×10^6 IU m^{-2} rIL-2 was significantly ($P = 0.029$) higher (nearly twice as high) than the AUC(0,24 h) after administration of (20×10^6 IU m^{-2}) in one daily cumulative dose.

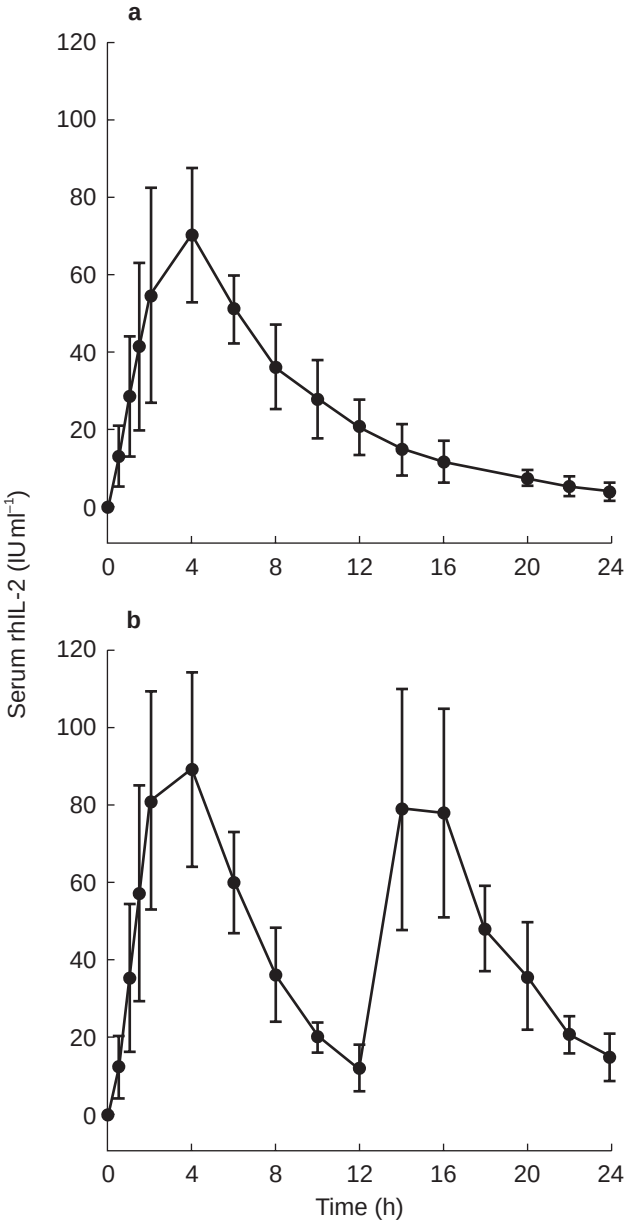

Figure 1 Pharmacokinetics of rhIL-2 in patients with metastatic renal cell cancer treated subcutaneously. Serum levels of rhIL-2 were measured with ELISA and are expressed as mean ± s.d. (a) Mean levels in seven patients receiving 20×10^6 IU m^{-2} s.c. as single injection. (b) Mean levels in three patients receiving 10×10^6 IU m^{-2} s.c. twice every 12 h.

Soluble IL -2 receptor (sIL -2R)

The serum concentrations of the sIL-2R of all 10 patients are shown in Figure 2. Prior to immunotherapy three patients exhibited normal sIL-2R levels (25–115 pmol l^{-1}), while seven patients had elevated levels, which ranged from 182 to 828 pmol l^{-1}. There was no significant difference between both study groups prior to the treatment ($P=0.425$), 24 h after the first injection of s.c. rhIL-2 ($P=0.425$) and 48 h after start of therapy ($P=0.275$). During rhIL-2 therapy the concentrations of the sIL-2R increased during the first 48 h continuously, but not significantly, in treatment group A from 286 pmol l^{-1} up to 614 pmol l^{-1} ($P=0.237$) and in treatment group B from 325 pmol l^{-1} up to 955 pmol l^{-1} ($P=0.109$). Three days after s.c. administration of rhIL-2 the sIL-2R seemed to increase distinctly (group A: from 286 pmol l^{-1} up to 1168 pmol l^{-1} and group B: from 325 pmol l^{-1} up to 1934 pmol l^{-1}). The sIL-2R was measured only in two patients of each treatment group up to 3 days after the beginning of rhIL-2 therapy, therefore we could not calculate significance using the Wilcoxon test. When summarizing both study groups we could show that the sIL-2R increased significantly ($P=0.016$) 72 h after the beginning of rhIL-2 treatment.

We also evaluated the influence of the increase of sIL-2R concentrations on the values of AUC(0,24 h). During the first 48 h after rhIL-2 administration, the increase of the sIL-2R had no apparent influence on the AUC values. After more than 48 h, there was a trend towards a decrease in AUC in those patients with sIL-2R concentrations over 1200 pmol l^{-1} ($n=3$), although this failed to reach statistical significance. The serum levels of sIL-2R and rhIL-2 of one typical patient who received 10×10^6 IU m^{-2} rhIL-2 s.c. are shown in Figure 3.

Discussion

In this study we have determined the pharmacokinetics of two different dose regimens of subcutaneous rhIL-2 administration in patients with metastatic renal cell cancer. Since ELISA assays have been employed, the current results do not necessarily correspond to the availability of biologically effective IL-2. We have shown for the first time that administration of 10×10^6 IU m^{-2} rhIL-2 s.c. twice daily (every 12 h) results in a significantly higher ($P=0.029$) total AUC(0,24 h) than s.c. administration of 20×10^6 IU m^{-2} once daily. Although pharmacokinetic parameters are best

Table 1 Pharmacokinetic parameters of a single injection of rhIL-2 at 20×10^6 IU m^{-2} s.c. in seven patients with metastatic renal cell cancer.

Number	t_{max} *(h)*	C_{max} *(IU ml^{-1})*	$t_{½,z}$*(4,24 h)* *(h)*	*AUC(0,12 h)* *(IU ml^{-1} h)*	*AUC(0,24 h)* *(IU ml^{-1} h)*
1	2.0	105	3.1	647	748
2	4.0	80	4.8	649	829
3	6.0	45	6.5	375	501
4	4.0	52	5.3	366	450
5	4.0	66	6.2	483	657
6	4.0	75	5.0	583	732
7	4.0	80	4.9	401	450
Mean ± s.d.	4.0 ± 1.2	72 ± 20	5.1 ± 1.1	501 ± 125	627 ± 153

Table 2 Pharmacokinetic parameters of two consecutive injections of rhIL-2 at 10×10^6 IU m^{-2} s.c. every 12 h in patients with metastatic renal cell cancer.

Number	*Time (h)*	t_{max} *(h)*	C_{max} *(IU ml^{-1})*	$t_{1/2,z}$*(4,12 h)* *(h)*	*AUC (IU ml h^{-1})*
8	0–12	4.0	67	2.9	446
9		4.0	116	2.1	698
10		4.0	83	3.5	585
Mean ± s.d.		4.0 ± 0	89 ± 25	2.8 ± 0.7	576 ± 126
8	12–24	2.0	48	3.5	369
9		4.0	88	3.1	555
10		2.0	110	3.6	737
Mean ± s.d.		2.7 ± 1.7	82 ± 31	3.4 ± 0.3	554 ± 184
	0–24				576 ± 554 = 1130

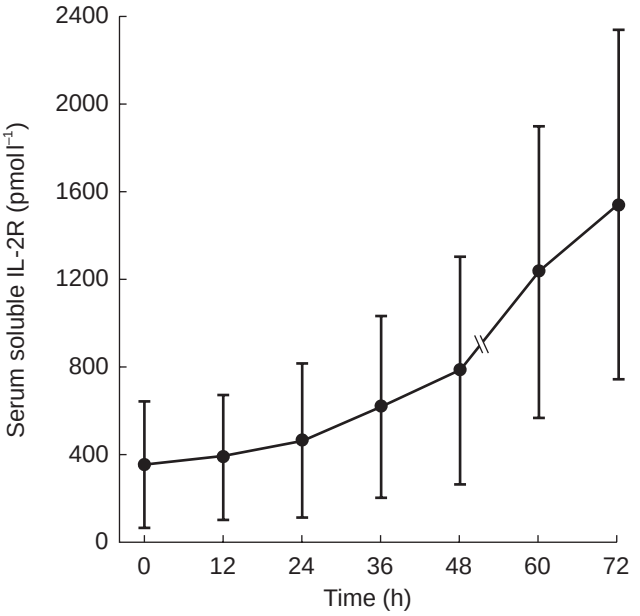

Figure 2 Serum concentrations (mean ± s.d.) of soluble IL-2 receptor measured by ELISA during rhIL-2 therapy ($n = 6$ until 48 h, $n = 4$ until 72 h).

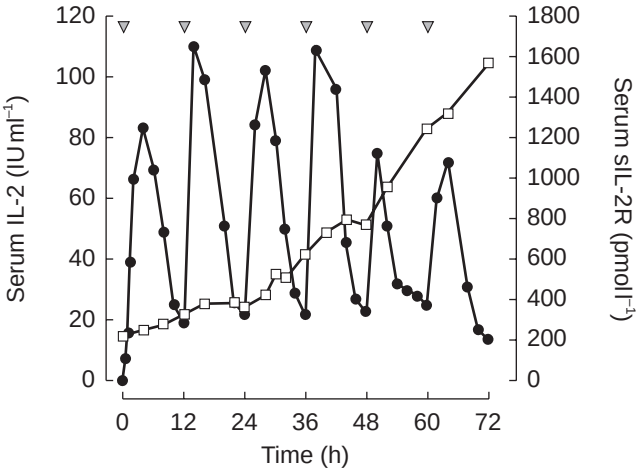

Figure 3 Serum levels of rhIL-2 (●) and soluble IL-2 (□) receptor after subcutaneous application (10×10^6 IU m^{-2} s.c. every 12 h for 3 days, ▼) in one typical patient.

determined from i.v. bolus and infusion data there are several reports of IL-2 pharmacokinetics following i.m., i.p. and s.c. administration [8–10]. Until now no previous study described the pharmacokinetic data of different dose regimens of subcutaneous rhIL-2 administration. Only Ettinghausen *et al.* [20] demonstrated that intraperitoneal (i.p.) injection of rhIL-2 three times a day was more effective than the cumulative IL-2 dose administered daily i.p. or i.v.

Furthermore, we could show, that there was no significant difference between the C_{max} ($P = 0.208$) and AUC(0,12 h) ($P = 0.305$) values after administration of a single dose of 10×10^6 or 20×10^6 IU m^{-2} rhIL-2 s.c. Gustavson *et al.* [10] reported that serum concentrations of rhIL-2 following i.v. administration ($0.1–30 \times 10^6$ U) increased in an apparently dose-proportional manner. However, when administered s.c. ($0.1–3.0 \times 10^6$ U), the increase in serum concentration was less than expected, which may have been due to a dose-dependent reduction in bioavailability for s.c. administered rhIL-2 [10]. We suggest that this effect is caused by an incomplete release of rhIL-2 from the subcutaneous injection site, hence, administration of doses higher than 10×10^6 IU m^{-2} rhIL-2 did not lead to significantly increased bioavailability. This observation is consistent with the results of other studies [21, 22] whereby recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) was administered s.c. in different doses. Stute *et al.* [21] described that C_{max} and the AUC did not increase proportionally to the dose of s.c. rhGM-CSF; to explain this phenomenon a reduced absorption from the injection site was also hypothesized [21].

Our pharmacokinetic parameters $t_{1/2} = 2.8–5.1$ h and $t_{max} = 4.0$ h are compatible with previous pharmacokinetic studies [9, 10]. Konrad *et al.* [9] used Cetus units (1 Cetus unit = 6 IU); they reported after i.v. bolus administration of a median dose of 20×10^6 IU m^{-2} rhIL-2 a nearly two-fold higher AUC (2465 IU ml^{-1} h) when compared with the present AUC after s.c. rhIL-2 administration of 20×10^6 IU m^{-2} in two daily doses (10×10^6 IU m^{-2} every 12 h) in our study (AUC(0,24 h) = 1130 IU h ml^{-1}). Comparing s.c. with i.v. bolus administration, the peak levels after s.c. administration were more than 10–100 times lower than immediately after i.v. bolus rhIL-2, but were approximately constant for several hours before gradually decreasing. Therefore, the reported toxicity of i.v. rhIL-2 was much higher in comparison to the s.c. application route [9].

We found elevated sIL-2R levels in seven of 10 patients with renal cell carcinoma prior to rhIL-2 therapy. Elevated sIL-2R levels have been described earlier in patients with advanced renal cell carcinoma [23]. Furthermore, sIL-2R levels are increased in several diseases, mostly in those of

infections [24, 25] or neoplastic character, like malignant melanoma, multiple myeloma, chronic myelogenous leukaemia [23, 26, 27].

After 3 days of s.c. rhIL-2 therapy the sIL-2R concentrations increased significantly ($P=0.016$). During the first 48 h after the start of rhIL-2 treatment the sIL-2R seemed to have no influence on the AUC values, because of the low sIL-2R concentrations. But in parallel to the increase of the sIL-2R concentrations over 1200 pmol l^{-1} a tendency toward reduced AUC amounts could be observed. A potential immune modulatory role of the sIL-2R has been discussed earlier [28], but not without controversy [29, 30]. The main objection against such a role was the low affinity (K_d: 10 nmol l^{-1}) of the sIL-2R for IL-2, which is 1000-fold lower than the binding affinity of the membrane-bound heterotrimeric receptor complex (K_d: 0.01 nmol l^{-1}) [16–18, 30–32]. Nevertheless, inhibition of IL-2 driven effects like proliferation of IL-2 dependent mouse CTLL cell line or inhibition of induction of cell-mediated cytotoxicity has been demonstrated by several investigators [30, 33–35].

In addition to the *in vitro* studies, in several clinical studies the putative physiological significance of sIL-2R has been investigated. During systemic administration of rIL-2 in humans, elevated sIL-2R levels have been observed earlier [15]. We described previously that during long-term s.c. rIL-2 treatment both soluble and cell surface IL-2R (CD25) exhibit a significant increase [15]. We could observe a positive correlation between the serum levels of sIL-2R and CD25 cell surface expression on peripheral blood lymphocytes. The quantitative correlation stated for soluble and membrane-bound IL-2R expression may be explained by its subsequent shedding without the transmembrane domain of the CD25 molecule [32].

The exact mechanism of the immune modulatory capacity of sIL-2R *in vitro* and *in vivo* remains still to be clarified. Here, we suggest two different possible mechanisms of immune modulation by the soluble IL-2R depending on its concentration. *In vitro* we could previously demonstrate that the neutralizing capacity for IL-2 driven immune responses (like CTLL-proliferation, cytotoxicity to malignant cell lines) is dose-dependent for IL-2R concentrations up to 100 pmol l^{-1} and is not due to a direct functional interaction between sIL-2R and free IL-2; at this lower concentration, an interaction of sIL-2R with the membrane-bound IL-2R seemed to be possible [18].

Our present *in vivo* data showed a trend towards decreasing AUC(0,24 h) values for sIL-2R concentrations higher than 1200 pmol l^{-1}. Because of the low affinity of sIL-2R in comparison to the membrane-bound IL-2R, a direct interaction between the sIL-2R and free nonbound IL-2 seemed to be possible only at higher concentrations of the sIL-2R.

In conclusion, the pharmacokinetics of s.c. rhIL-2 in patients with metastatic renal cell carcinoma can be summarized as follows:

[A] Split s.c. injection (every 12 h) of rhIL-2 results in a significantly ($P=0.029$) higher total AUC(0,24h) than corresponding single dose administered once daily. [B] There were no significant differences between the C_{max} and AUC(0,12h) levels after single s.c. administration of 10×10^6 and 20×10^6 IU m^{-2} of rhIL-2, respectively. [C] AUC(0,24h) following s.c. administration of rhIL-2 in two daily doses was comparable with AUC(0,24h) reported after i.v. bolus rhIL-2. [D] The sIL-2R increased significantly ($P=0.016$) during treatment with rhIL-2, and soluble IL-2R levels over 1200 pmol l^{-1} seemed to cause a decrease of AUC(0,24h).

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