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METASTATIC RENAL CELL CARCINOMA TREATED WITH PURIFIED LEUKOCYTE INTERFERON

Clinical response in relation to tumor DNA content

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Abstract

Twenty-eight patients with renal cell carcinoma and evaluable metastases were treated with a purified leukocyte interferon $2-7 \times 10^6$ IU daily. DNA content was analyzed by flow cytometry. Nine patients had lung metastases only, 12 had dissemination to lungs and to other sites as well, and 7 had metastases in other sites only. Two patients had complete response and one had minor response of lung metastases. Another 3 patients had mixed responses: 2 of them complete disappearance and one minor response of lung metastases but concomitant progression of skeletal and abdominal metastases. The overall response rate restricted to lung metastases only was 21% (6 of 28 patients) including the 3 patients with mixed response. The 6 patients with any kind of response were found among 19 patients with aneuploid primary tumors while no response was noted in 8 patients with diploid tumors.

Key words: Renal cell carcinoma, metastases, interferon, DNA content.

Metastatic renal cell carcinoma is known to be highly resistant to cytostatic and radiation therapy (1). Hormonal treatment such as medroxyprogesterone acetate has been reported to give objective responses in a low percentage but most responses are incomplete and of short duration (2). Spontaneous regression has been reported in renal cell carcinoma more frequent than in other malignant tumors (3), suggesting that host factors may control tumor progression. Numerous recent studies have confirmed that interferon has therapeutic effect in patients with metastatic renal cell carcinoma (4–7) with response rates mostly in the range of 10–25%. Both natural and recombinant interferons have been used. The routes of administration and dose schedules have varied. A serious draw-back so far of the native leukocyte interferon has been its low degree of purity, hampering direct comparisons with the recombinant interferons.

The aim of this phase II study was to evaluate clinical efficacy as well as side-effects of a highly purified human native interferon in the treatment of metastatic renal cell carcinoma. Since tumor DNA content significantly predicts survival in patients with metastatic renal cell carcinoma (8) the aim was also to study a possible relationship between response to treatment and tumor DNA content.

Material and Methods

Patients. Thirty patients with metastatic renal cell carcinoma were treated with purified leukocyte interferon at the Department of Oncology, University Hospital, Umeå, between January 1985 and March 1988. There were 23 men and 7 women, mean age 60 years (range 34–78). For evaluation of therapy response the following inclusion criteria were used: histologically verified diagnosis of renal cell carcinoma with evidence of metastases, measurable size of the metastases, no previous interferon therapy and at least 2 months' progression of the disease prior to therapy. Two patients were excluded from the evaluation of therapy response due to too short a treatment period, in one of them interferon was discontinued because of increasing icterus after 3 days treatment and in the other interferon was discontinued due to inacceptable toxicity

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after 2 weeks' treatment. Twenty-seven of the 28 patients evaluable for response were operated upon with perifascial nephrectomy. Sixteen patients had received previous therapy, 15 with medroxyprogesterone acetate and one with cytostatics. At the time of the nephrectomy or diagnosis 5 patients were in stage I according to Robson et al. (9), 7 in stage II–III while 16 had known metastatic disease (stage IV). The median time between nephrectomy and appearance of metastases for the 12 patients with stage I–III disease was 18 months (range 3–76). The median time between diagnosis of metastases and start of interferon treatment was 13.9 months (range 2–36 months) and 7.4 months (range 2–26 months) for patients with diploid and aneuploid tumors respectively. At the start of interferon treatment the patients had a Karnofsky index ranging between 60 and 100 with a median of 80. The mean Karnofsky index for patients with diploid tumors and those with aneuploid tumors were 78.8 and 78.4 respectively. The study was approved by the ethics committee of the University of Umeå.

Follow-up and evaluation of response. All patients were followed at least every second month with clinical and radiological examinations as indicated by known disease manifestations. Evaluation of response was performed according to criteria recommended by WHO (10): complete response (CR)—complete disappearance of all lesions determined by 2 observations not less than 4 weeks apart; partial response (PR)—partial decrease by at least 50% in the sum of the products of the largest diameters of all measurable lesions, no lesions should have progressed and no new lesions appeared; stable disease (SD)—no significant change for at least 2 months; progressive disease (PD)—increased size with more than 25% of the sum of the products of the largest diameters. In addition, minor response (MR) was defined—decrease of the metastases by at least 25 up to 50% in the sum of the products of the largest diameters of all measurable lesions. All radiologic examinations were reevaluated with regard to response. Toxicity was graded according to WHO critera (10). The analysis included follow-up of all patients until April 1990.

Interferon preparation. For each production, batch buffy coats from several hundred individuals were pooled and interferon was produced following the Cantell procedure (11). After the last ethanol precipitation step the material was further purified using a combination of sequential immunoaffinity and conventional chromatography (unpublished). The final product was tested by SDS-PAGE after addition of human serum albumin. The protein bands were in the molecular range of 18–27 kD and they all showed immunoreactivity with a rabbit anti-human interferon-Á antiserum (Fig. 1). As no protein band with negative reaction was detected of the immunoblot the material was judged to be more than 90% pure. The specific activity was 2×10^8 U/mg protein. The material

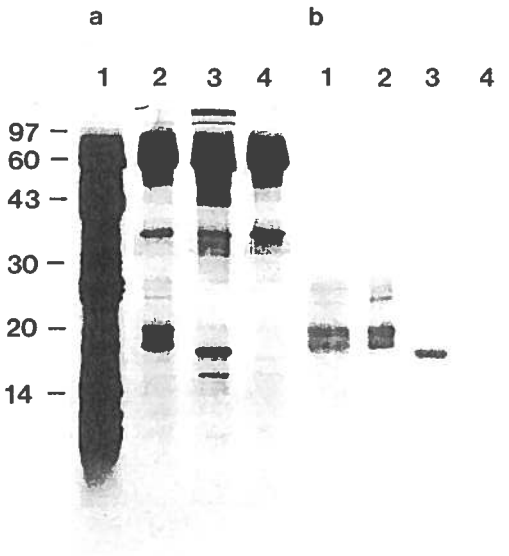

Fig. 1. SDS-polyacrylamide gel electrophoresis (a) and immunoblotting (b) of human leukocyte interferon. The SDS-PAGE was silver-stained and a ploYclonal rabbit antiserum was used in the immunoblotting experiment. 1) Cantell preparation of human leukocyte interferon, starting material for the purification procedure; 2) interferon, alfa-native preparation, purified as described in 'Material and Methods'; 3) recombinant interferon-α2; 4) human serum albumin, which was added to all samples.

was tested by *Limulus*-test and contained less than 1 ng/ml of LPS. It was checked that the purification method did not allow survival of added human immunodeficiency virus. All buffy coats were tested for the absence of HBsAg and HIV antibodies. Interferon was assayed by an ELISA method or by a virus replication inhibition method (12). The material was calibrated against the international reference preparation 69/19, using an internal laboratory standard.

Interferon therapy. Interferon (Interferon Alfa Native, BioNative AB, Umeå, Sweden) was given as a daily dose of 2×10^6 U subcutaneously. Therapy was continued as long as there were no obvious signs of progression and an acceptable toxicity. The aim was to give interferon at least for 2 months before a definite decision was made whether to continue or to stop the treatment. In patients with progressive disease the dose was escalated to 4×10^6 IU in 3 and to 7×10^6 IU in another 3 patients.

DNA analysis. The method of flow cytometric DNA anlysis has been described in detail previously (13). Briefly, from each tumor, 8 fresh tumor and one kidney cortex sample were minced and stained using propidium iodide according to Vindeløv (14). After staining, the samples were filtered and run in a FACS Analyzer or a FACScan (Becton-Dickinson, Sunnyvale, CA, USA). In 13 patients without fresh tumor tissues, 2–3 paraffin-embedded tissue samples from the primary tumors were cut and deparaffinized. Isolation of the nuclei was performed according to Schutte et al. (15). After filtration the nuclei were

stained with the same propidium iodide solution as used for the fresh material. The tumor samples were denominated diploid (DNA index 1.0) when only one peak was detected and aneuploid when 2 separate peaks were found, since it was assumed that all tumor samples contained normal as well as tumor cells. A primary tumor was denominated diploid when all tumor samples were diploid and accordingly a tumor was aneuploid when at least one of the samples was aneuploid.

Results

Response. Nine of the patients had lung metastases only, while 12 had dissemination to the lung and to other sites as well. Seven patients had metastases in other sites than the lungs. The effect of treatment according to metastatic sites is shown in Table 1. Response was only seen in the lungs. Two patients had CR and one had MR of multiple lung metastases. The 2 patients with CR of multiple lung metastases were both previously treated with medroxyprogesteron acetate but had then progressive disease. Another 3 patients had mixed responses with in 2 patients complete disappearance and in one minor response of multiple lung metastases but concomitantly progression of skeletal and abdominal metastases. Thus, 6 of the 28 patients (21%) showed response when including the 3 with mixed responses. The median time to any response was 3.5 months (range 1–7 months). However, objective responses according to the strict WHO-criteria could only be documented in 2 of patients (7%). The duration of these objective responses were 11 and 14 months respectively. None of the patients showed any response though escalation of the interferon doses.

DNA content. Eight of the 28 patients had diploid primary tumors and 19 had aneuploid primary tumors while DNA analysis was unsuccessful in one (Table 1). DNA analysis was performed on tissue from metastases in 5 of the patients. In 4 of these metastases the DNA content corresponded to that found in at least one sample of the primary tumor (one diploid and 3 aneuploid tumors). In one patient the metastasis had a DNA-index at 1.7 while the DNA-index was 1.3 in the primary tumor. There was no therapy response in any of 8 patients with diploid primary tumors but 3 of them had stable disease. All the 6 patients with any kind of response were found among the 19 patients with aneuploid primary tumors. In addition 3 patients with aneuploid tumors had stable disease.

Survival. The survival time after appearance of metastases for patients with diploid and aneuploid tumors respectively is shown in Fig. 2. Three patients were alive with progressive disease after 27, 28 and 29 months respectively. The survival time after diagnosis of metastases was significantly longer for patients with diploid primary tumors than for those with aneuploid primary tumors ($p = 0.036$, log-rank test). There was no statistically significant difference in survival time after the start of treatment between

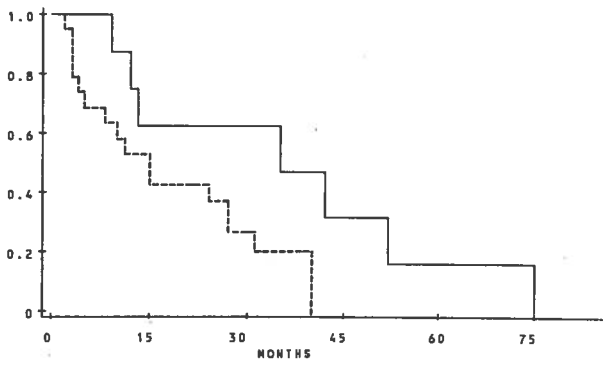

Fig. 2. Survival curves comparing outcome of 8 patients with diploid (——) and 19 patients with aneuploid (– – –) primary tumors respectively. The survival is calculated from the first appearance of metastases. $p = 0.036$.

Table 1

Response to interferon treatment according to metastatic sites and to DNA content of primary tumors in 28 patients with renal cell carcinoma

Response	Lung	Lung and other site	Other site than lung	Total	DNA content of the primary tumor		Total
					Diploid	Aneuploid	
CR	2	2*	–	4	0	4	4
PR	–	–	–	0	–	–	0
MR	1	1*	–	2	0	2	2
SD	3	3**	1	7	3	3	6#
PD	3	6	6	15	5	10	15
Total	9	12	7	28	8	19	27#

* mixed responses with response only in lung and progressive dissease in other sites.
** stable disease in lung, progressive disease in other sites.
# DNA analysis was unsuccessful in one patient.

Table 2

Side-effects according to WHO toxicity scale during treatment with purified leukocyte interferon in 30 patients with metastatic renal cell carcinoma

Signs and symptoms	Grade			n	%
	1	2	3		
Transient flu-like symptoms	4	7	–	11	33
Liver (transaminases, icterus)	2	2	1*	5	17
CNS (lethargy, depression)	1*	1*	1*	3*	10
Nausea/vomiting	–	2	–	2	7
Leukopenia	–	2	–	2	7
Hemolytic anemia	–	–	1*	1*	3

* The symptoms were not clearly related to treatment.

patients with diploid and aneuploid tumor respectively ($p = 0.30$, log-rank test).

Toxicity. All patients were evaluable for toxicity. Initial transient flu-like symptoms were seen in 11 of the 30 patients (Table 2). These symptoms were associated with fever $< 38°C$ (WHO grade 1) in 4 patients and fever 38°C–40°C (WHO grade 2) in 7 patients. In all cases the fever reaction subsided within 4 days. Transient clinically insignificant leukopenia (WHO grade 2) was seen in 2 patients and reversible rise of transaminases WHO grade 1 in 2 patients and WHO grade 2 in 2 patients. One patient, with liver metastases had a rapid increase of bilirubin and the therapy was discontinued after only 3 days. No thrombocytopenia was noted. One patient, a 64-year-old woman with lung metastases, had evidence of hemolytic anemia (WHO grade 3) after 5 months of interferon treatment. The anemia was irreversible upon interruption of therapy and did not respond to corticosteroids. The patient died of cardiovascular complications after splenectomy. Two patients had severe CNS symptoms (grade 2 and 3 respectively) with lethargy, depression and in one patient with confusion. These symtoms, however, could not be clearly related to interferon treatment. One of these patients had evidence of cerebral atrophy on CT scan and the other had a depression probably on constitutional basis. In both patients the mental status was unchanged or worsened after discontinuation of interferon therapy. Interferon treatment was interrupted after short-term treatment due to toxicity in 2 patients; one of them had severe nausea and vomiting and the other severe icterus (WHO grade 3).

Discussion

Renal cell carcinoma has been resistant to a number of therapeutic approaches (1, 2). The response rates to interferon treatment have ranged from 5 to 31% in individual studies (4–7) with an overall rate of about 13%. In the present study a low dose of purified leukocyte interferon was used. The objective response rate in the present study according to the strict WHO-criteria was only 7% which is rather low compared with the best results reported. The interferon dosage in our trial was low and reviews of reported studies suggest a suboptimal effect of both low and high-dose regimens while the best results have been reported from trials using a moderate dose level (4). However, additional 7% of the patients in our study had mixed response with complete disappearance of lung metastases but with concomitant progression at other metastatic sites. This might indicate heterogeneity of the tumor cell populations in different metastic sites or difference in host factors at the metastatic sites, e.g. leading to different bioavailability of interferon. The selection of patients may greatly influence the response rate.

Obvious response was in our study restricted to metastases in the lung, which also previously has been reported as the most frequent response site (4–7). In a previous study we have showed a significantly higher frequency of lung metastases in patients with aneuploid tumors as compared with those with diploid primary tumors (13). Interestingly, in the present study all patients with any response to interferon treatment had aneuploid primary tumors while none of the patients with diploid tumors responded. This association between response and DNA content was not found in a recent study by Fosså et al. (16) but in that study only one sample from each tumor was analyzed. There was, in the present study, a significant difference in survival time between patients with diploid and aneuploid primary tumors in concordance with previous results in patients with metastatic renal cell carcinoma (8,17). In the present study, patients with aneuploid tumors had a mean survival time of 17.4 months compared with about 6 months for patients with no interferon treatment in the previous studies (8, 17). However, this difference in survival time might well be explained by the selection of the patients for this study.

In our study a more than 90% pure preparation of leukocyte interferon was used, containing 10–15 proteins with interferon activity. No proteins devoid of interferon activity were found. Differences in response rates and spectrum of side-effects in different studies may be at least partly due to differences in doses used and in administration schedules. One of our patients had evidence of hemolytic anemia. Previously a few cases of hemolytic anemia associated with interferon treatment have been reported. One of these cases developed recurrent hemolytic anemia after repeated interferon treatment (18), suggesting a major pathogentic role of interferon. The relatively low frequency of side-effects, especially flue-like symptoms, in the present study, was most likely due to the low dose of interferon used.

In conclusion, in our study only aneuploid tumors responded to treatment with purified leukocyte interferon

and the response was restricted to lung metastases. The results suggest that analysis of DNA content might be of value for the design of randomized, prospective trials in patients with metastatic renal cell carcinoma.

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REFERENCES

1. deKernion JB, Berry D. The diagnosis and treatment of renal cell carcinoma. Cancer 1980; 45: 1947–56.
2. Hrushesky WJ, Murphy GP. Current status of the therapy of advanced renal adenocarcinoma. J Surg Oncol 1977; 9: 277–88.
3. Katz SE, Schapira HE. Spontaneous regression of genitourinary cancer—an update. J Urol 1982; 128: 1–4.
4. Krown SE. Interferon treatment of renal cell carcinoma. Current status and future prospects. Cancer 1987; 59: 647–51.
5. deKernion JB, Sarna G, Figlin R, Lindner A, Smith RB. The treatment of renal cell carcinoma with human leucocyte alfa-interferon. J Urol 1983; 130: 1063–6.
6. Fujita T, Asano H, Naide Y, et al. Antitumor effects of human lymphoblastoid interferon on advanced renal cell carcinoma. J Urol 1988; 139: 256–9.
7. Muss HB, Costanzi JJ, Leavitt R, et al. Recombinant alfa interferon in renal cell carcinoms: A randomized trial of two routes of administration. J Clin Oncol 1987; 5: 286–91.
8. Ljungberg B, Roos G, Stenling R. Deoxyribonucleic acid content in metastatic renal cell carcinoma—clinical implications. Semin Surg Oncol 1988; 4: 165–8.
9. Robson CJ, Churchill BM, Andersson W. The results of radical nephrectomy for renal cell carcinoma. J Urol 1969; 101: 239–301.
10. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47: 207–14.
11. Cantell K, Hirvonen S, Koistonen V. Partial purification of human leucocyte interferon on a large scale. Methods Enzymol 1981; 78: 499–505.
12. Armstrong JA. Semi-micro, dye binding assay for rabbit interferon. Appl Environ Microbiol 1971; 21: 723–5.
13. Ljungberg B, Stenling R, Roos G. Tumor spread and DNA content in human renal cell carcinoma. Cancer Res 1988; 48: 3165–7.
14. Vindeløv LL. Flow microfluometric analysis of nuclear DNA in cells from solid tumors and cell suspensions. Virchows Arch [B] 1977; 24: 227–42.
15. Schutte B, Reyners MMJ, Bosman FT, Blijham GH. Flow cytometric determination of DNA ploidy level in nuclei isolated from paraffin-embedded tissue. Cytometry 1985; 6: 26–30.
16. Fosså SD, Nesland JM, Melvick JE, Jacobsen AB, Moe B. Prediction of objective response to recombinant interferon-alpha with or without vinblastine in metastatic renal cell carcinoma. Acta Oncol 1990; 29: 303–6.
17. Mukamel E, Ritchie A, Hannah J, Loverkovich D, deKernion JB. The prognostic significance of the DNA content of renal cell carcinoma. J Urol 1989; 139: 293.
18. Denes A. Severe autoimmune hemolytic anemia associated with interferon alpha 2-B in a patient with renal cell carcinoma. Blood 1987; 70 (suppl 1): 108.