Effects of Interferon on Tumor Tissue Content in Liver Metastases of Human Carcinoid Tumors

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ABSTRACT

In 21 patients ultrasound-guided cutting biopsies, from carcinoid metastases of the liver, were taken before and after therapy with α-interferon. Each biopsy was examined under light microscopy and the amount of tumor tissue and connective tissue was quantified and then correlated to objective response to interferon therapy. A significant reduction of the amount of tumor tissue, in spite of unaltered metastatic size and a corresponding increase in connective tissue, was seen after interferon therapy. A more pronounced reduction of tumor tissue occurred after long-term interferon therapy. A positive correlation between objective therapy response and tumor tissue reduction was also present. Patients responding poorly, or not at all, to therapy did not show any significant decrease in tumor tissue.

Since treatment with immune response modifiers is expected to increase in the near future, it is important to choose the right investigations for therapy monitoring, and since all patients in this investigation had unchanged tumor size on repeated radiological examinations, it is obvious that microscopic examination of core biopsies is a better method for evaluating effects of long-term therapy than tumor size measurement with radiological techniques. Further, the results may indicate that interferon exerts a cytotoxic effect on carcinoid tumor cells in vivo.

INTRODUCTION

In patients with metastatic spread to the liver from tumors derived from the neuroendocrine cell system, interferon therapy has been promising and is now routinely used in our unit (1, 2). The antineoplastic mechanisms of action of α-interferon are not fully known. α-Interferon has demonstrated inhibitory effects on oncogene expression, DNA replication, and protein synthesis (3–5). Tumor cell division is blocked mainly in the G0/G1 phase (6). α-Interferon has, however, not yet been shown to possess a definite cytotoxic effect on tumor cells.

To monitor therapy effects in patients with liver metastases, CT, MRI, and US have been used frequently (7). A decrease in tumor size has been used as a sign of effect of treatment, while unchanged or increased size has been considered to indicate stable disease and progression, respectively (8). In patients treated with interferon for liver metastases from neuroendocrine tumors, positive clinical response and decrease in tumor markers are often present despite unchanged tumor size as measured with various radiological techniques (9). This indicates that tumor size cannot be used as a single indicator of therapy response in patients receiving interferon treatment.

Preliminary data, obtained by routine histopathological examination of liver biopsy specimens from carcinoid patients, have indicated that an increased amount of connective tissue and a corresponding decrease in tumor tissue is present in biopsies taken after interferon therapy. An investigation was performed in order to determine whether this phenomenon could be verified by quantitative determination of the relative amount of tumor tissue in individual metastatic nodules.

MATERIALS AND METHODS

Twenty-one different patients with untreated liver metastases from mid-gut carcinoids were investigated before and after the start of interferon therapy. There were 13 men and 8 women; the mean age was 61.5 years (range, 36–83 years). At least one ultrasound-guided liver biopsy, in which tumor tissue was obtained, before the start of therapy and one or more biopsies taken after various intervals (3, 6, 12, or 24 months) after initiation of therapy were obtained in all cases. The same metastasis was biopsied in 17 of the patients, although this was not possible because of technical reasons in 4. Interferon was given as human leukocyte interferon in 18 patients and recombinant interferon (Intron A) in 3. The leukocyte interferon consisted of a mixture of 16 α-interferons with a specific activity of 1 x 10^6 IU/mg of protein. The recombinant interferon was a pure interferon α-2b with a specific activity of 1 x 10^6 IU/0.006 mg of protein.

Tumor biopsies were taken, under ultrasound guidance and using an automatic sampling technique (10, 11), with a Biopsy-Cut needle (Bard Urological Division of C. R. Bard, Covington, GA) with an external outside diameter of 1.2 or 2 mm. The specimens were divided and fixed in 10% buffered formalin and Bouin's fluid, dehydrated, and embedded in paraffin. Sections, about 4 µm thick, were cut and stained with hematoxylin-eosin, van Gieson's stain, the argentaffin reaction of Masson, and the argyrophil reactions of Grimelius (12).

The argyrophil reaction of Grimelius was applied to establish the neuroendocrine origin of all tumor specimens used for the study and the argentaffin reaction of Masson complemented with immunocytochemistry to document the presence of serotonin in the tumor cells. For immunocytochemical analysis, monoclonal serotonin antibodies (Mas 055, clone YC5/45.HLK; SeraLab Limited, Crawley Down, Sussex, England) were used to visualize serotonin-containing endocrine tumor cells (11).

For a quantitative investigation the hematoxylin-eosin-stained material was viewed, without any knowledge of clinical or laboratory data, in a light microscope at x160 using a ocular with a squared 21 x 21 matrix grid. For each biopsy the type of tissue (tumor tissue, connective tissue or other tissue elements) at each crossing point was registered in five different fields, which was considered sufficient since the biopsies were rather uniform in the distribution of tumor and connective tissue. From these data the percentage of tumor tissue, for each specimen, could be calculated by dividing the number of points crossing tumor tissue with the total number of points crossing any tissue element. The amount of tumor tissue was expressed as a percentage of the total tissue amount and the connective tissue percentage was calculated in a similar way.

The values obtained before the start of interferon therapy were then compared to those obtained after initiation of interferon therapy. A total of 53 biopsy specimens from 21 different patients were examined with the quantitative technique. For statistical analysis a linear regression analysis was performed for evaluation of the decrease in tumor tissue over time. In 10 patients more than two biopsies were performed, and both a quadratic function and a linear were used. Since the quadratic function did not give a significantly better curve fit, the linear regression was used for the whole material. Two-tailed, paired Student's t test was used for analysis of tumor tissue content before and after therapy. The biopsies before the start of therapy was compared with the biopsy after therapy in each patient regardless of the time of...
therapy. For comparison of tumor reduction to therapy response, Student's t test analysis was performed when tumor tissue reduction and tumor marker levels (plasma levels of neuropeptide-K and urinary levels of 5-hydroxy indole acetic acid) after interferon therapy were compared.

Objective tumor response was defined as a more than 50% reduction of tumor markers. Stable disease was defined as a less than 50% reduction of tumor markers with absence of new metastases. Progressive disease was defined as more than 25% increase in tumor markers or the development of new metastases (2, 13).

All patients underwent CT and US, and in two cases also MRI, at approximately the same time as the biopsy samples were taken, and the size and number of the metastases were recorded.

RESULTS

The specimens taken before initiation of therapy (n = 21) showed a mean amount of tumor tissue (Table 1) of 64.3% (range, 14–87%) and the connective tissue index was 35% (range, 13–86%). The corresponding values for specimens obtained after 3–24 months of interferon therapy (n = 32) were 38% (range, 0–87%) for tumor tissue and 56% (range, 13–100%) for connective tissue. The difference between tissue content before and after therapy was statistically significant (P < 0.05) using Student's t test.

The largest proportion of tumor tissue in the posttherapy group was seen in specimens obtained after 3 months (n = 4) of interferon therapy showing a mean tumor content of 53% (Table 1, Fig. 1). Specimens taken 6 (n = 11), 12 (n = 9), or 24 (n = 8) months after the start of therapy contained significantly smaller amounts of tumor tissue and showed a corresponding increase in connective tissue content (Figs. 1 and 2). The statistical analysis of the change in tumor tissue content with time showed a mean decrease in tumor tissue content of 2.5%/month (regression coefficient, −2.5 ± 1.3) with 95% confidence, indicating a statistically significant decrease in tumor tissue over time. If only patients with an objective therapy response are included, the resulting confidence interval was −3.6 ± 1.9.

A significant decrease in tumor tissue after therapy was present in patients with objective tumor response (n = 14) (P = 0.001), while patients with stable or progressive disease (n = 7) did not show any significant decrease in tumor tissue content after interferon therapy.

Statistical analysis of connective tissue content showed an almost exact mirror image of the tumor tissue analysis and is therefore not discussed in detail.

DISCUSSION

Recently α-interferon has been shown to possess potent therapeutic effects on neuroendocrine tumors with distant spread, i.e., liver metastases (1, 2). The antineoplastic mechanisms of action of α-interferon are not fully known. α-Interferon has demonstrated inhibitory effects on oncogene expression, DNA replication, and protein synthesis (3–5). Tumor cell division is...
carcinoid tumors exhibited a significantly decreased amount of effect was obtained after intraarterial infusion of streptozotocin, within 30 min after intraarterial infusion directly into the tumor an alkylating cytotoxic drug (15). A recent study we could observe a release of stored hormones on tumor cells has not yet been proven for α-interferon, but in a study in which human osteosarcomas in nude mice were treated similar phenomenon has been reported in an experimental tumor phenomenon since increase in connective tissue in other sites has not been described in patients receiving interferon. A similar phenomenon has been reported in an experimental study in which human osteosarcomas in nude mice were treated with α-interferon. In this study the tumor tissue was either completely or partly replaced by bone and marrow tissue of normal appearance after interferon therapy, while the tumor mass was unchanged in size. It has also been shown previously that tumor volume, evaluated with CT, MRI, and US, is unaltered during interferon therapy (9). Therefore tumor volume cannot be used to evaluate whether a therapy effect is present or not. For this reason light microscopic examination of biopsy specimens, in combination with radiological imaging techniques, at present seems to be the most discriminative method for evaluation of long-term therapeutic results in patients with liver metastases of carcinoid tumors.

REFERENCES

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