

**ENHANCEMENT OF TAMOXIFEN-INDUCED SUPPRESSION OF INSULIN-LIKE
GROWTH FACTOR I GENE EXPRESSION AND SERUM LEVEL
BY A SOMATOSTATIN ANALOGUE**

Hung Huynh and Michael Pollak*

**Departments of Medicine and Oncology,
Lady Davis Research Institute of the Jewish General Hospital and McGill University,
Montreal, Quebec, Canada H3T 1E2**

SUMMARY: Mitogenic responsivity of many neoplasms to IGF-I has been detected in a variety of *in vivo* and *in vitro* experimental systems. This has led to the proposal that pharmacological reduction of IGF-I bioactivity might represent a novel non-cytotoxic palliative therapy. We recently reported that tamoxifen, a commonly used antiestrogen antineoplastic agent, significantly suppresses IGF-I gene expression and serum IGF-I levels. We report here that the somatostatin analogue octreotide, previously demonstrated to reduce acromegalic levels of IGF-I towards normal, decreased serum IGF-I to $70 \pm 4\%$ (mean \pm SD) of control values and hepatic IGF-I expression to $65 \pm 10\%$ of control values in a short-term non-acromegalic rat model. Tamoxifen reduced serum IGF-I to $74 \pm 12\%$ of control values and hepatic IGF-I expression to $46 \pm 9\%$ of control values in this model, but the combination of octreotide and tamoxifen reduced serum IGF-I concentration to $49 \pm 10\%$ of control values and hepatic IGF-I gene expression to $12 \pm 9\%$ of control values. The levels of serum IGF-I and hepatic IGF-I gene expression were significantly less in animals treated with the combination of octreotide and tamoxifen than in animals treated with either agent alone ($p < .01$). This combination represents a novel pharmacological strategy for suppressing IGF-I gene expression that may be relevant to the design of clinical trials.

Insulin-like growth factor I (IGF-I) bioactivity in neoplastic tissue and target organs for metastasis is determined by factors including circulating IGF-I concentration, local IGF-I gene expression, and local and systemic concentrations of IGF binding proteins. In view of emerging evidence (reviewed in (1-4)) that a significant subset of human malignancies is mitogenically responsive to IGF-I, there is increasing interest in testing the hypothesis that pharmacological measures that reduce tissue IGF-I bioactivity would be therapeutically useful. Additional motivation for research concerning pharmacological methods to suppress IGF-I gene expression comes from data showing that in

*Corresponding author (fax: 514-340-8302).

experimental systems the inhibitory effect of dietary restriction on neoplastic proliferation is mediated by suppression of IGF-I levels (5). Specific compounds that are receiving attention as suppressors of IGF-I bioactivity include somatostatin analogues, growth hormone releasing hormone antagonists, growth hormone antagonists, antiestrogens, IGF binding proteins, antagonists to the type I IGF receptor, and suramin (6-10). Gene therapy approaches have also received attention in this regard (11).

Our clinical observation that tamoxifen suppresses serum insulin-like growth factor I (IGF-I) levels (7) has been confirmed in several subsequent studies (3,12-15). In laboratory investigations, we have obtained evidence for two mechanisms that may explain this action of tamoxifen. First, there is evidence that tamoxifen attenuates the pulsatile release of growth hormone (GH) by the pituitary gland (16,17), an action that would be expected to reduce GH-dependent hepatic IGF-I gene expression, and thereby reduce serum IGF-I concentration. A second mechanism involves a direct inhibitory effect of tamoxifen on IGF-I gene expression, which is detectable in liver as well as other tissues (18).

Somatostatin analogues such as octreotide (19) are known to dramatically reduce the elevated growth hormone secretion and thereby diminish the abnormally high IGF-I levels seen in acromegalic patients (20). These compounds also have a modest but statistically significant suppressive effect on normal levels of IGF-I found in non-acromegalic subjects (6). Recently, evidence has been presented that the somatostatin analogue octreotide, like tamoxifen, has direct (GH-independent) inhibitory effects on hepatic IGF-I gene expression (21). Somatostatin analogues have antiproliferative activity in certain experimental cancer models (22,23), and proposed mechanisms of action include direct effects mediated by somatostatin receptors on neoplastic cells, and indirect actions such as suppression of the growth hormone-IGF-I axis. There are several proposed mechanisms by which the combination of a somatostatin analog and an antiestrogen might achieve an additive antiproliferative effect (2,16), and reports of enhancement of the antineoplastic effects of a somatostatin analogue when co-administered with a LHRH analogue (23,24) provide a precedent for combination therapy involving somatostatin. In the present study, we sought to determine if the tamoxifen-induced reduction of IGF-I gene expression and serum IGF-I levels in female rats is enhanced by the co-administration of the somatostatin analogue octreotide (19).

Materials and Methods

We administered vehicles alone, tamoxifen, octreotide, or the combination of tamoxifen and octreotide to 50 day old female Sprague-Daley rats (Charles River, St. Constance, Quebec, Canada) as described in figure legends. Each treatment group comprised 6 animals. Following treatments, animals were sacrificed and samples of serum were obtained for radioimmunoassay of IGF-I following acid chromatography to remove IGF binding proteins, and livers were removed for extraction of RNA and quantification of IGF-I gene expression by Northern blotting, as previously described (18). The doses selected were those that gave maximal response as single agents in terms of serum IGF-I level (data not shown).

Results and Discussion

Figure 1 shows a representative Northern blot of hepatic IGF-I expression of a single rat from each experimental group. Figure 2A provides quantitative analysis of hepatic IGF-I expression in each treatment group, obtained by densitometrically scanning the sum of the 1.0, 1.4, and 7.5 kb IGF-I

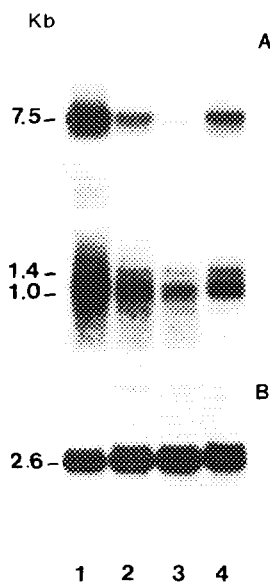
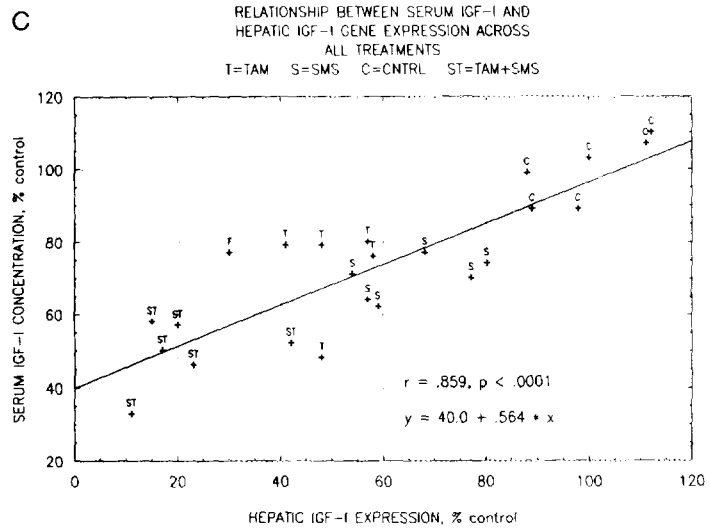
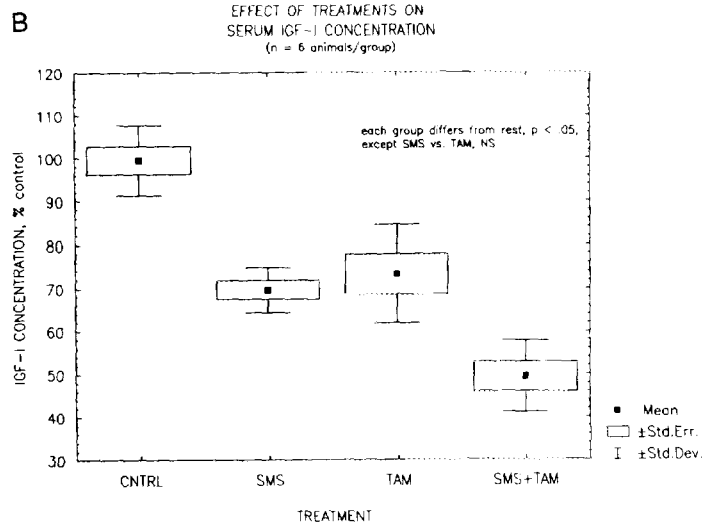
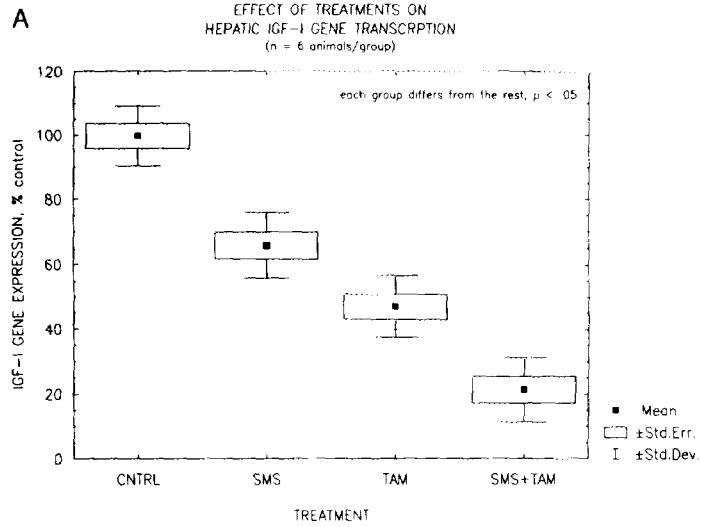


Figure 1. Representative Northern blots of IGF-I mRNA expression in intact rats treated with vehicle (lane 1), octreotide (lane 2), tamoxifen plus octreotide (lane 3), and tamoxifen (lane 4). Tamoxifen (10 mg/rat) was administered using a dose and route as described (18). On day 8 following tamoxifen administration, rats were either administered saline or 25 µg octreotide subcutaneously every 2 hours for 8 hours. Each experimental group consisted of six rats and the experiments were repeated three times with similar results. Following sacrifice, liver tissue was sectioned into small pieces and frozen in liquid nitrogen for Northern analysis. Total hepatic RNA was isolated and Northern blots were performed as previously described (18). Blots were hybridized either with (A) rat IGF-I cDNA (27) or (B) human β-actin (28). The Northern blot clearly shows maximum reduction of IGF-I in lane 3, which corresponds to the combined octreotide - tamoxifen treatment. Quantification of results is presented in Figure 2.

transcripts from the Northern blot of each individual animal, and plotting the mean, SEM, and standard deviation for each treatment group. The results of radioimmunoassays of serum pre-processed by acid chromatography to remove IGF binding proteins are given in Figure 2B, and Figure 2C relates IGF-I serum level and hepatic mRNA abundance to treatment for all experimental animals. The experimental treatments had no significant effect on serum IGF binding protein concentrations as estimated by densitometric scanning of ligand blots (data not shown).

The data demonstrate that in our experimental system, a significantly greater reduction in IGF-I gene expression and serum level is achieved by the combination of octreotide and tamoxifen than by either agent alone. The high correlation between hepatic IGF-I expression and serum IGF-I level ($r=0.859$, $p<0.0001$) is consistent with the fact that the liver is the major source for circulating IGF-I. In view of the strong evidence for IGF-I responsivity of human breast cancer cells, it is possible that the IGF-I suppressive effect of tamoxifen contributes to the antiproliferative effect of the drug (26). If this is the case, the octreotide - tamoxifen combination described here may exhibit greater antineoplastic activity than tamoxifen. It remains to be determined if the enhancement of tamoxifen-induced suppression of IGF-I gene expression by octreotide seen in our animal model also occurs in humans on long-term treatment. Nevertheless, our results motivate evaluation of the combination in preclinical

Figure 2. Quantification of effect of octreotide (SMS), tamoxifen (TAM), and tamoxifen-octreotide combination (SMS+TAM) on serum IGF-I levels and hepatic IGF-I expression. Animals were treated as described in the legend to figure 1. [A] Effect of treatments on hepatic IGF-I gene expression. Mean, standard deviation, and standard error of the mean density of bands corresponding to IGF-I mRNA transcripts for each treatment group are presented. Individual Northern blots were performed for each of the 6 animals in each experimental group. Octreotide decreased hepatic IGF-I gene expression relative to the control by $35\pm 10\%$, tamoxifen by $54\pm 9\%$, and the combination by $88\pm 9\%$. Values for each treatment group were significantly different from each other and the control value ($p < .01$ for each comparison, Mann-Whitney U test). [B] Effect of treatments on serum IGF-I concentration. Mean, standard deviation, and standard error of the IGF-I levels for each treatment group are presented. Individual radioimmunoassays were performed on serum samples from each of the six animals per experimental group. Blood samples were collected by cardiac puncture and the serum was processed to remove IGF binding proteins as described (18). The mean control serum IGF-I concentration was 1250 ± 120 ng/ml. Tamoxifen (TAM) reduced serum IGF-I by $26\pm 12\%$, octreotide (SMS) by $30\pm 4\%$, and the combination of TAM and SMS by $51\pm 10\%$. The serum IGF-I concentration was significantly less in animals treated with combination of octreotide and tamoxifen than in animals treated with either agent alone ($p<0.01$, Mann-Whitney U-test). [C] Scatterplot relating hepatic IGF-I gene expression and serum IGF-I levels for each experimental animal. The abbreviations used in identifying the points are: C, control; T, tamoxifen-treated; S, octreotide-treated; ST, octreotide and tamoxifen treated. There was a significant correlation between hepatic IGF-I gene expression and serum IGF-I levels ($r=0.859$, $p<0.0001$).



tumor model systems and clinical trials. Indeed, the results reported here provide a novel rationale for ongoing clinical research studies which are comparing the efficacy of tamoxifen to that of tamoxifen combined with octreotide in advanced breast cancer.

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