Effect of Interferon-α Treatment on [^{68}Ga-DOTA,Tyr3,Thre8] Octreotide Uptake in CA20948 Tumors: A Small-Animal PET Study

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In peptide receptor radionuclide therapy of neuroendocrine tumors, improvements have been made by increasing the affinity for receptors and by protecting critical organs (e.g., kidneys). However, tumor parameters involved in radiopeptide uptake are still under investigation. Interferon-α (IFNα) is used as biotherapy for neuroendocrine tumors. Several mechanisms of action are described, but the potential effect of IFNα on tumor uptake of labeled peptide has not been studied in vivo yet. **Methods:** Twenty-six male CA20948 tumor–bearing Lewis rats were imaged before and during IFNα treatment using quantitative small-animal PET with [^{68}Ga-DOTA,Tyr^3,Thre^8]octreotide. Imaging was performed at days 0, 3, and 7. Animals were divided into 3 groups according to the treatment: control (injected daily with saline), half (4 d of IFNα treatment from day 0 to day 3, then saline), and full (7 d of IFNα). A daily dose of IFNα (1.5 mIU) was administered subcutaneously. Quantitative PET results are expressed as percentage injected dose per cm^3 and normalized to baseline (day 0) values. Tumor size was monitored by PET and caliper measurements. **Results:** Gross tumor uptake and tumor volumes increased in all groups over the 7-d period. On day 3, mean ± SD ratios to day 0 were 1.2 ± 0.2, 1.3 ± 0.5, and 1.2 ± 0.4, respectively, for control, half, and full groups. On day 7, respective values were 1.1 ± 0.2, 1.3 ± 0.6, and 1.5 ± 0.4. At day 3, a comparison among groups showed no statistically significant difference. At day 7, the full group showed a significantly higher ratio in activity concentration than the control group ($P = 0.021$). A good correlation was found between tumor volumes assessed by small-animal PET and caliper measurements ($R = 0.89$, $P < 0.0001$). **Conclusion:** As expected, over a period of 7 d, both tumor volumes and radiopeptide uptake increased in all animals. However, the activity concentration increased significantly more at day 7 in animals treated for 7 d with IFNα, compared with controls. This is the first, to our knowledge, in vivo indication that IFNα is able to increase tumor uptake of the labeled analog in a small-animal model of neuroendocrine tumors. The mechanisms underlying this effect (flow, vascular permeability, receptor upregulation) remain unknown and need to be further investigated.

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Most neuroendocrine tumors express a high density of somatostatin receptors (sstrs), allowing the use of this property for diagnostic and therapeutic purposes. Current therapeutic approaches toward gastroenteropancreatic neuroendocrine tumors include surgery, chemoembolization, chemotherapy, and biotherapy using somatostatin analogs or interferon-α (IFNα) (*1,2*).

Peptide receptor radionuclide therapy (PRRT) is another therapeutic opportunity that takes advantage of the high sstr expression of tumors to target them with β-emitter–labeled somatostatin analogs. Clinical results showed response in 6%–30% of the patients (*3*). Several procedures have been developed to increase therapy efficiency by protecting critical organs, mainly the kidneys (*4*). However, tumor parameters involved in radiolabeled analog uptake and their potential modulation remain to be investigated.

Introduced in 1983 as therapy for neuroendocrine tumors (*5*), IFNα proved effective, with a symptomatic response in 40%–60% of the patients. Tumor reduction, however, was obtained in only 10%–15% of the patients (*6*). Although some data suggest a cumulative effect of IFNα and somatostatin analogs, because of the lack of evidence current clinical practice does not recommend a combination treatment (*7*). Some data suggest that IFNα could induce an upregulation of sstrs (*7,8*), but, to the best of our knowledge, no original article has clearly demonstrated this effect.

Assuming that the amount of receptors at the cell surface limits the peptide uptake, further upregulation of receptors on tumor cells could be a key for optimization of PRRT.

This study aimed to evaluate the effect of IFNα on tumor uptake of the ^{68}Ga-labeled analog [^{68}Ga-DOTA,Tyr^3,Thre^8] octreotide using small-animal PET in the CA20948 rat model of pancreatic neuroendocrine tumor. The ability of

PET quantitative imaging to follow the same animal over time was exploited to compare the tumor uptake in each animal after experimental treatment with its own baseline uptake values.

MATERIALS AND METHODS

Study Design

To compare the increase of [^{68}Ga-DOTA,Tyr^3,Thre^8]octreotide uptake in tumors, animals were randomly assigned to 3 groups, according to the IFNα treatment: control (no IFNα treatment, subcutaneous injection of 100 μL of saline daily), half (subcutaneous injection of IFNα for 4 d, then saline for 3 d), and full (subcutaneous injection of IFNα for 7 d). Imaging was performed on days 0 (baseline imaging just before the first injection of IFNα or saline), 3, and 7. The general design of the treatment and imaging studies is depicted in Figure 1.

Animals and Tumor Inoculation

Twenty-eight male Lewis rats (age, 7 wk) were subcutaneously injected with 1 mL (~10^8 cells/mL) of tumor cell suspension prepared with crude unfrozen CA20948 tumor tissue in Dulbecco's modified Eagle's medium plus GlutaMax-1 (Invitrogen Corp.).

The CA20948 neuroendocrine exocrine pancreatic tumor cell line was previously well characterized (*9*). It was shown to strongly express sstr 2 at the cell surface; this expression was quantitated, and the binding of sstr analogs in vivo was shown to be receptor-specific (*9,10*). In addition, this cell line internalizes several ^{111}In-labeled somatostatin analogs and was used previously to demonstrate the effect of PRRT (*11*).

Rats were purchased from Charles River Ltd. Tumors were allowed to grow for 15–20 d after inoculation before study. Animals were housed 2 or 3 per cage and fed ad libitum. All imaging procedures were performed under continuous isoflurane anesthesia (induction, 3%; maintenance, 1.5%; Forene [Abbott Laboratories Ltd.]). Animals were housed in a facility approved by the Belgian Ministry of Agriculture in accordance with current regulations and standards. The experimental design was approved by the Ethics Committee on animal experimentations of the Medical School of the Université Catholique de Louvain. The principles of laboratory animal care (*12*) were strictly followed.

[^{68}Ga-DOTA,Tyr^3,Thre^8]Octreotide

[DOTA,Tyr^3,Thre^8]octreotide was provided by Biosynthema. The ^{68}Ge/^{68}Ga generator was obtained from Cyclotron Ltd. Elution was performed with 0.1 M ultrapure HCl (prepared from ultrapure HCl 30% TraceSelectUltra and Ultrapure water; Fluka). Labeling was performed by adding 2 μg of peptide and 8 μL of 2.5 M Na acetate to 150 μL of eluate containing 30–90 MBq of ^{68}Ga activity and by heating the solution for 10 min at 80°C, as described previously (*13*). The reaction solution was then cooled in ice-cold water for 5 min. Five microliters of ethylenediaminetetraacetic acid (5 mM) were added to chelate any residual ^{68}Ga. Saline was added to reach a final volume of 600 μL; the resulting solution was used for 2 animals, each of which received 1 μg of peptide and approximately 15–45 MBq of ^{68}Ga. Radiopharmaceutical purity was assessed by high-performance liquid chromatography (HPLC) using a C18, 150/4.6 Nucleosil 100-5 column and HPLC-grade acetonitrile as a solvent (Chromanorm for HPLC—Gradient Grade; Macherey-Nagel) and was in excess of 90% for all experiments.

IFNα Treatment

IFNα (Intron A; Schering-Plough) was injected subcutaneously once a day at 1.5 mIU (~4–5 mIU/kg). All animals, including those in the control group, received paracetamol in drinking water (1 mg/mL). Rats were weighed daily, and tumor dimensions were measured by the caliper method (product of 2 largest diameters). The surface measurements (S) obtained using the caliper method were fitted with:

$$S = aV^b, \qquad \text{Eq. 1}$$

where V is the volume of interest (VOI; as determined by PET), and parameters a and b are not constrained.

Image Acquisition

PET images were acquired with the Mosaic (Philips) scanner. Activity was measured in all syringes before and after injection using a dose calibrator. Immediately after tracer injection, a transmission scan was acquired in single mode using a 370-MBq ^{137}Cs source for attenuation correction. A short scan for emission contamination correction was obtained thereafter. A 15-min emission scan was started about 25 min after injection of [^{68}Ga-DOTA, Tyr^3,Thre^8]octreotide. At the end of the emission scan, recovery from anesthesia was reached within 5 min in all cases.

Reconstruction and Quantification of Uptake

Transmission data were reconstructed after emission contamination correction. Raw data were corrected for attenuation, random, and scatter coincidences and for system dead-time. All images were reconstructed with a fully 3-dimensional row-action maximum likelihood algorithm. Each reconstructed matrix was composed of 120 transverse 128 × 128 images with voxels of 1 mm^3.

VOIs were drawn manually on tumors by the same operator using PMOD software (version 2.75; PMOD Technologies Ltd.). VOI statistics were decay-corrected and converted to injected dose

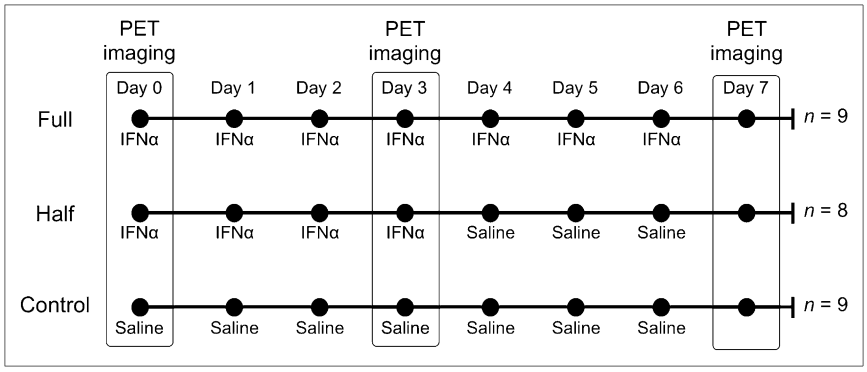

FIGURE 1. Design of study.

using an external standard for calculation of percentage injected dose (%ID) in tumors. Results are expressed as %ID/cm^3 after correction for VOI. Uptake data were normalized to day 0 for each animal.

Statistical Analysis

Unless otherwise stated, data are presented as mean ± SD. The Student *t* test, paired Student *t* test, Pearson correlation test, or nonlinear regression was used, as appropriate. The Shapiro–Wilk normality test was applied to assess gaussian distribution in small groups. All tests were performed using Prism software (version 5.0; GraphPad Software).

RESULTS

Effect of IFNα Treatment

No rat receiving IFNα treatment presented limiting toxicity that precluded the continuation of the study. One rat was excluded from the control group because of technical failure, and 1 rat from the full group was excluded because of death due to tumor growth. In the control, half, and full groups, 9, 8, and 9 rats, respectively, could be fully analyzed. Over a period of 7 d, rats in all groups showed a reduction in body weight ranging between 0.5% and 16%. The mean rat weight on day 0 was 340.0 ± 34.5, 318.8 ± 27.6, and 331.3 ± 31.7 g, respectively, in control, half, and full groups. On day 7, weight was 321.9 ± 39.5, 296.4 ± 34.3, and 317.9 ± 31.0 g, respectively. No statistically significant difference was found among the groups at any day (Student *t* test, $P > 0.11$; all groups successfully passed the Shapiro–Wilk normality test).

Tumor Uptake After IFNα Treatment

All tumors displayed a relatively heterogeneous uptake at all times. Tumor uptake, expressed as %ID/cm^3, is reported in Table 1. No statistically significant difference among the groups for %ID/cm^3 values was found at any time. In the control group, a statistically significant difference was noted between day 0 and day 3 (paired *t* test, $P = 0.007$), whereas in the fully treated group, a statistically significant difference was observed between day 0 and day 7 (Wilcoxon matched-pair test, $P = 0.01$) and between day 3 and day 7 (Wilcoxon matched-pair test, $P = 0.02$).

TABLE 1
%ID/cm^3 of Tumor Tissue Measured by PET in 3 Groups at Days 0 (baseline), 3, and 7

Group	Day 0	Day 3	Day 7
Control	0.50 ± 0.11	0.60 ± 0.17*	0.55 ± 0.11
Half	0.50 ± 0.13	0.58 ± 0.16	0.60 ± 0.17
Full	0.45 ± 0.17	0.50 ± 0.10	0.64 ± 0.17*,†

*$P < 0.05$ vs. day 0.
†$P < 0.05$ vs. day 3.
Data are mean ± SD.

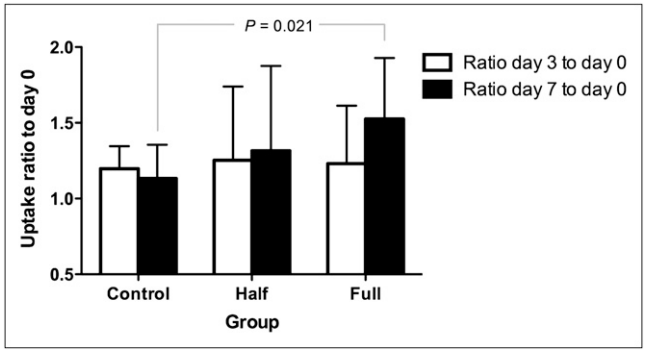

FIGURE 2. Mean ± SD ratios to day 0 of activity concentration (%ID/cm^3) at days 3 and 7. Statistically significant difference between control and full groups is observed at day 7.

The ratio of uptake to day 0 was computed to compare each animal with its own baseline values. On day 3, ratios to day 0 were 1.2 ± 0.2, 1.3 ± 0.5, and 1.2 ± 0.4, respectively, for control, half, and full groups. On day 7, respective values were 1.1 ± 0.2, 1.3 ± 0.6, and 1.5 ± 0.4. All groups passed the Shapiro–Wilk normality test. At day 3, the comparison among groups showed no significant differences. At day 7, a statistically significant difference was found between the control group and the fully treated group ($P = 0.021$, Fig. 2). In addition, only the full group showed a statistically significant increase in concentration between day 3 and day 7, as assessed by the respective ratios to day 0 ($P = 0.013$).

Tumor Volume After IFNα Treatment

Tumor volume data are shown in Figure 3. Tumor volumes increased significantly in all groups over time (paired Student *t* test, $P < 0.01$ in all cases). On day 0, VOIs were 10.9 ± 3.7, 10.8 ± 5.1, and 13.4 ± 3.5 cm^3, respectively, in control, half, and full groups. On day 3, volumes were 16.8 ± 6.5, 14.8 ± 4.0, and 19.0 ± 5.4 cm^3, respectively, and on day 7, volumes were 24.8 ± 11.5, 19.7 ± 7.3, and 25.8 ± 8.5 cm^3, respectively. No statistical difference in VOIs was

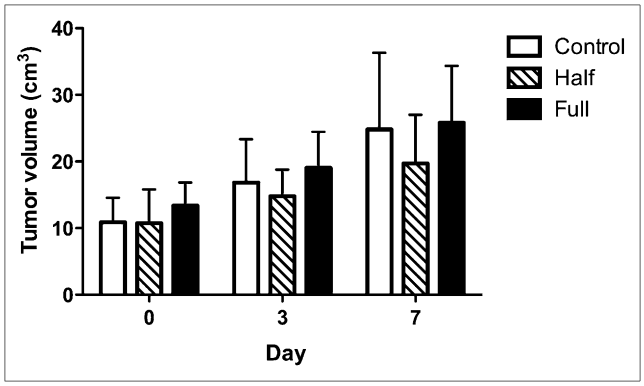

FIGURE 3. Mean ± SD tumor volumes assessed by small-animal PET VOIs at each imaging session. No statistically significant difference between groups was found.

noted among groups, whatever the day (Student t test, $P >$ 0.09 in all cases).

A nonlinear regression correlation between volume drawing (VOIs) and tumor surface determined by the caliper method was fitted to Equation 1 and gave a value for S of 1.28 $V^{0.65}$ ($R = 0.89$, Fig. 4).

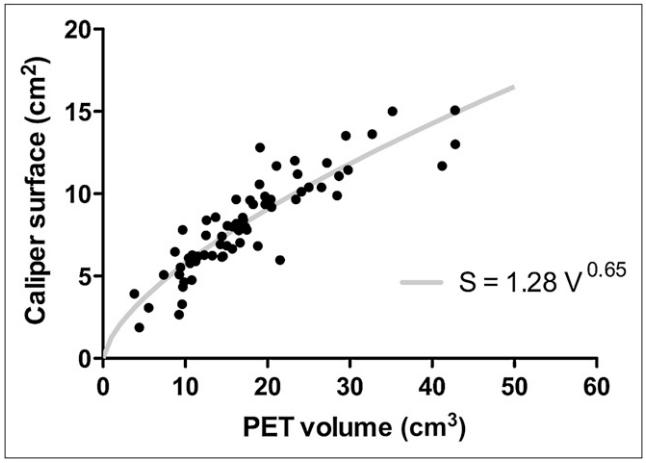

FIGURE 4. Nonlinear correlation between tumor volumes assessed by small-animal PET and caliper measurements (2 largest perpendicular diameters). $R = 0.89$.

DISCUSSION

This study demonstrates that IFNα administered over 7 d affects the uptake of the ^{68}Ga-labeled somatostatin analog [DOTA,Tyr^3,Thre^8]octreotide in the CA20948 animal model of pancreatic neuroendocrine tumors. The quantitative capability of PET allowed uptake in the animal to be followed over time, using day 0 imaging of each animal as its own baseline value.

Although this kind of experiment cannot fully explain all mechanisms involved, it has the double advantage of reduction of the number of animals in a longitudinal pharmacologic study and direct observation of the sum of all potential effects. Conversely, it does not allow the dissection of the possible involved mechanisms, which requires appropriate additional investigations.

Because of the significant variance in tumor size at the 3 studied time points (i.e., days 0 [baseline], 3, and 7), the gross uptake (expressed as %ID/cm^3) did not differ with statistical significance among groups. However, when rescaling all data to the baseline value (which is somehow an intrinsic tumor feature unaffected by the potential therapeutic effect of IFNα), we noted a significant increase in uptake at day 7, but only in the fully treated group.

Effects of IFNα have been previously studied, and different mechanisms of action were reported. IFNα interacts with specific cell-surface receptors inducing transcription of several genes (*14–17*). Observed effects of IFNα are fibrosis of the tumor (*18*), antiangiogenic effect by inhibiting transcription of the vascular endothelial growth factor gene (*19,20*), induction of apoptosis (*6,21*), and blockade of cell cycle (*22*). IFNα was also reported to increase the expression of some receptors, such as class I antigens (*23*), urokinase-type plasminogen activator receptor (*24*), and epidermal growth factor receptor (*25*), at the cell surface.

In clinical practice, combination of biotherapies using IFNα and somatostatin analogs is somewhat controversial. In vitro, a clear additive inhibitory effect was demonstrated on pituitary adenoma cells (*26*). In patients, although some studies do not show evidence of improved response by combination therapies (*6,7,27*), others advocate such combinations in individual cases (*2,23,28,29*).

In this study, we investigated IFNα used alone with the goal of optimizing PRRT, by potentially increasing the analog uptake in tumor cells. We cannot definitely infer from these data that the expression of sstrs is upregulated, but the final objective—that is, increased uptake of the labeled analog—was observed.

A few tumor parameters involved in peptide uptake have been studied so far. In vitro, upregulation of sstrs was observed after chronic exposure to cold octreotide (*30*). This effect was confirmed by preclinical studies and proved to be present only after prolonged or chronic exposure and not after single administration of the analog (*31,32*). In addition, upregulation of sstrs was shown in rats treated with suboptimal doses of ^{177}Lu-[DOTA,Tyr^3,Thre^8]octreotide (*33*). IFNα was described as increasing the density of sstrs at the cell surface (*7,8*), but—to the best of our knowledge (PubMed search)—this increase was reported only as a cumulative effect of IFNα and somatostatin analogs on tumor cells. We found no original article demonstrating specifically the upregulation of sstrs by IFNα treatment either in vivo or in vitro. Further, the cellular or biochemical mechanisms involved remain entirely unexplained.

We can conclude from the current study that ours is the first data indicating that IFNα increases somatostatin analog uptake in a model of neuroendocrine tumors. As for every experimental protocol, potential biases inherent to the animal model should be discussed.

First, it is impossible to predict with certainty to what extent the animal data can be extrapolated to humans. Clinical studies should be undertaken to address this issue by comparing the uptake of the radiolabeled analog in IFNα-treated patients before and during treatment. In addition, human-recombinant IFNα was used here to treat rats, and species dependency may influence results observed in humans in a similar setting. Third, uptake was assessed by the concentration of labeled peptide in tumors, namely, the activity divided by the volume (%ID/cm^3). Tumor volumes were determined by manually drawn VOIs around the tumor, and this bias might be a source of error. However, as a double-check test, we found an excellent correlation ($R = 0.89$) between the PET volume and 2-dimensional caliper measurements used as an accepted reference method. More convincing, however, was the fact that the best fit gave a value of b close to two thirds, which is the theoretic value

to translate a volume into a surface. Fourth, the dosage was based on doses used in rats for pharmacologic studies as reported in the literature (*34*). The duration of the treatment was intrinsically limited by the natural growth of CA20948 tumors. As a matter of fact, baseline imaging has to be performed on tumors that have already reached a sufficient volume for accurate delineation and reduction of the partial-volume effect (i.e., tumors ~1 cm in diameter); conversely, the last imaging session has to be performed before tumors reach unacceptable sizes according to the basic standards for humane handling of laboratory animals (i.e., ~3–4 cm in largest diameter) (*35*).

Cellular or vascular microenvironment mechanisms involved in this increased uptake concentration are yet unknown. Further research is required to understand the relationship between IFNα treatment and increased uptake, and several hypotheses have to be investigated.

First, whether the expression of sstrs is upregulated after treatment with IFNα can be evaluated by binding assays ex vivo on tumors grown in vivo. This procedure is arduous because in such a model, the role of heterogeneity and necrosis must be considered for data analysis as they may be confounding. Second, increased uptake after IFNα could be specific to this tumor model, and repeating such experiments with another tumor line would be justified. The ideal tumor line would be one with neuroendocrine features expressing high levels of sstrs, but with a slower growing time that would allow a longer follow-up. Third, increased flow might be speculated to be a way to enhance peptide delivery to the tumor. However, an antiangiogenic effect of IFNα was demonstrated in several animal models (*36–38*) and in neuroendocrine tumors in humans (*20*). In addition, no increased blood flow was shown in patients with carcinoid tumors receiving chronic IFNα treatment (*39*). Conversely, IFNα was shown to increase blood flow only within 60–90 min after injection in a model of melanoma (*40,41*); this finding does not apply to our studies, in which the last IFNα injection was performed 24 h before the last imaging session, as illustrated in Figure 1. Finally, we have previously shown that blood flow in this tumor model cannot be investigated accurately by PET, because it proved to be low (*32*), thus definitively limiting the exploration of this avenue using noninvasive methods.

CONCLUSION

IFNα given daily for 7 d was shown to further increase [^{68}Ga-DOTA,Tyr^3,Thre^8]octreotide uptake in CA20948 tumors, as compared with control groups. This study can be considered as a proof of concept for future studies on the influence of IFNα on somatostatin analog tumor uptake and tumor-absorbed doses. The cellular mechanisms involved in this effect remain undetermined, and studies are needed to assess the dose–response relationship, effect on tumors in terms of absorbed doses in a therapeutic setting, and, finally, indication for IFNα before PRRT for neuroendocrine tumors using somatostatin analogs.

DISCLOSURE STATEMENT

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