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ORIGINAL ARTICLE

Effect of Octreotide LAR Dose and Weight on Octreotide Blood Levels in Patients With Neuroendocrine Tumors

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Objectives: Octreotide long acting repeatable (LAR) is widely used for the control of symptoms of functional neuroendocrine tumors. At doses of 30 mg/mo, up to 40% of patients require subcutaneous octreotide "rescue" and up to 40% of patients are given more than 30 mg of LAR/mo. Octreotide acetate binds to the sst2 receptor with an affinity (K_d) of approximately 1×10^{-9} mol/L (≅1000 pg/mL), but higher (≅10,000 pg/mL) concentrations of octreotide are required to completely saturate this receptor. Octreotide blood level measurement may be useful to guide LAR therapy in symptomatic patients or in patients who have tumor growth on traditional LAR doses. We hypothesize that LAR doses of 60 mg/mo will produce blood levels of 10,000 pg/mL or greater. At identical monthly LAR doses, patients with higher weights will require more medication to achieve similar plasma octreotide levels than individuals with lower body weights.

Methods: Trough plasma, serum, urine, and saliva octreotide levels were obtained from 52 patients with carcinoid syndrome receiving 20 (n = 8), 30 (n = 19), or 60 mg LAR/mo (n = 10). Octreotide levels were determined by radioimmunoassay.

Results: The mean ± SD plasma octreotide levels for patients receiving 20, 30, or 60 mg LAR/mo were 2518 ± 1020, 5241 ± 3004, and 10,925 ± 5330 pg/mL, respectively. Patient weight (kilograms) was inversely related to plasma octreotide levels. There was a significant correlation between plasma octreotide levels and octreotide levels measured in urine, saliva, and serum.

Conclusions: Frequent measurement of octreotide levels may be useful to guide octreotide therapy in patients with poorly controlled symptoms or those patients experiencing tumor growth.

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Octreotide acetate (Sandostatin®; Novartis Pharmaceutical, East Hanover, NJ) is an octapeptide congener of somatostatin that is widely used for the control of the symptoms associated with functionally active neuroendocrine tumors.[1] Octreotide acetate controls symptoms by inhibiting the secretion of peptides and amines associated with these functional tumors. Octreotide binds preferentially to sst2, 1 of a family of somatostatin receptors (sst1–sst5). Octreotide acetate binds to the sst2 receptor with an affinity (K_d, 50% receptor saturation) of approximately 1×10^{-9} mol/L (≅1000 pg/mL).[2,3] Nearly complete saturation of the sst2 receptor should occur with circulating drug levels approximately 10 times higher than the K_d (10^{-8} mol/L or ≅10,000 pg/mL). Subsequent inhibition of G-protein–associated signal transduction pathways block secretory vesicle exocytosis and the release of the target peptide or amine.

Introduced into clinical practice in the 1980s, octreotide is the only analog currently available in the United States for the treatment of neuroendocrine tumors.[1] Lanreotide, another sst2-preferring somatostatin-like peptide, with a similar sst2 binding affinity, is approved for the treatment of neuroendocrine tumors in Europe but it is not on the market in the United States. Octreotide is available in 2 forms: an immediate release form (aqueous) and a sustained release form (LAR). The current Food and Drug Administration (FDA)-approved indications for the use of octreotide include its use for the control of secretory diarrhea from carcinoid and vasoactive intestinal peptide (VIP)-omas and the control of growth hormone secretion in patients with acromegly. However, a wide variety of other "out of indication" uses for this drug have been proposed. Clinically, the LAR form is widely used for long-term symptom control, and the aqueous form of octreotide is commonly used as "rescue" medication for the acute control of symptoms.[4]

Octreotide LAR is currently available in 3 doses: 10, 20, and 30 mg. Dosing this medication is at the discretion of the physician; however, the recommended starting dose of this drug is 20 mg/mo.[5] Few, if any, physicians currently choose an alternative starting dose of LAR based on the patient's weight, height, body surface area, or body mass index (BMI). In the registration trials for this drug, up to 40% of patients required

rescue mediation several times a week, independent of the monthly LAR dose (10, 20, or 30 mg/mo) used for long-term symptom control.[3] This implies that circulating drug levels produced by current doses of this medication are inadequate to completely saturate sst2, and thus, the drug levels are not adequate to maximally suppress symptoms or tumor growth.[2,3] Recently, Anthony et al[6] showed that, in current clinical practice, 20% to 40% of patients received LAR doses higher than the FDA-approved maximal dose (30 mg/mo) to adequately control symptoms or to suppress tumor progression. The reasons why the individual physicians choose to use these higher doses were not investigated in this study.

Some investigators believe that octreotide has a suppressive effect on the growth of tumor cells in vitro and clinically has an antiproliferative or tumorostatic effect. Octreotide growth inhibitory action in tumor cells may be independent of its neurovascular functions. In a variety of in vitro studies, octreotide has been show to inhibit the growth of tumor cells and a variety of other sst2-expressing cell types such as retinal pigment epithelial cells and smooth muscle cells. Interestingly, in these in vitro studies, the antiproliferative effect of octreotide is biphasic. At concentrations of 10^{-12} mol/L or less, octreotide has little or no effect on cell growth. Increasing drug concentrations up to levels of 10^{-9} to10^{-8} mol/L is associated with concentration-dependent inhibition of cell growth. Maximum inhibition of cell growth is seen in the range of 10^{-9} to10^{-8} mol/L. Further increases in drug concentration ($>10^{-8}$ mol/L) are not associated with further increases in inhibition of cell growth, but rather, higher drug concentrations are associated with a dose-dependent loss of inhibition of cell growth compared with the10^{-9} to 10^{-8} mol/L concentrations of octreotide.[7–10] Thus, the optimum antiproliferative effects of octreotide occur in a relatively narrow concentration range, and under- or overdosing of this drug might lead to higher rates of tumor cell proliferation.

Octreotide has also been shown to have a direct effect on the growth of angiogenic vessels based on the unique expression of sst2 on proliferating, but not normal, blood vessels.[10,11] Thus, octreotide therapy, when administered at optimal doses, should have an antiproliferative effect on the tumor (at the cell level), the angiogenic blood vessels, or both. Gulec et al[11,12] recently reported on the use of nude mouse–human tumor xenografts (that contained either sst2-positive or sst2-negative tumor cells) in a unique tumor fragment-based in vitro angiogenesis assay. These authors showed that sst2-preferring radiolabeled somatostatin analogs could destroy sst2-expressing tumor cells and destroy their sst2-expressing angiogenic response. In contrast, sst2 nonexpressing tumor fragments were not destroyed by these sst2-preferring radiolabeled somatostatin analogs, but their angiogenic vessels (sst2 positive) were destroyed. These studies show that the sst2 receptor is uniquely expressed on angiogenic vessel endothelium. In contrast, endothelial cells in normal human blood vessel do not express sst2 receptors. This unique expression of sst2 in angiogenic blood vessels may make this receptor an excellent target for somatostatin-based therapies.

Theoretically, inadequate circulating levels of octreotide should be associated with poorer clinical symptom control and a higher rate of tumor growth, whereas blood levels that saturate sst2 should be associated with better clinical symptom control and better control of tumor growth rates. In the octreotide LAR drug registration trial, LAR doses of 10, 20, and 30 mg/mo were associated with mean plasma levels ($\pm$SD) of 1153 $\pm$ 748 (n = 21), 1914 $\pm$ 972 (n = 20), and 4247 $\pm$ 2733 pg/mL (n = 24), respectively.[5] These levels are in the suboptimal range for complete receptor saturation. In this study, approximately 40% of patients required the use of rescue medication (subcutaneous aqueous octreotide at doses of 100–500 μg 3 times a day) several days per week. Previous studies have examined the toxicity of extremely high doses of octreotide and lanreotide and found them to be safe. Doses of octreotide of 6 mg/d have been associated with few if any serious side effects. Thus, the upper limit of octreotide dosing is essentially unlimited by safety concerns.[13]

We hypothesized that the octreotide concentration in the plasma or serum would be directly proportional to the LAR dose. Furthermore, we hypothesized that, at identical monthly LAR doses, patients with higher weights would have lower plasma octreotide levels than individuals with significantly lower body weight. Finally, we hypothesized that 60 mg/mo doses of LAR would produce blood levels approaching or exceeding the concentration needed to saturate the sst2 receptor (≅10,000 pg/mL).

MATERIALS AND METHODS

To test these hypotheses, 52 patients with carcinoid syndrome, receiving octreotide LAR for control of symptoms associated with neuroendocrine tumors, had their plasma, serum urine, and saliva octreotide levels measured by a highly specific and sensitive radioimmunoassay developed at Inter-Science Institute (Inglewood, CA). The octreotide immunoassay is based on a 1-step, extraction-free, simultaneous assay procedure designed to quantify the analyte in serum, plasma, urine, and saliva. Briefly, octreotide standards (2–256 pg), quality control, or patient samples were dispensed into tubes containing 200 μL of phosphor-saline–based assay buffer, followed by the addition of 100 μL of octreotide tracer and 100 μL of a titered concentration of octreotide antiserum (Peninsula Laboratories, Division of Bachem, San Carlos, CA). After 24 hours of incubation at 4°C, 200 μL of appropriately diluted goat antirabbit IgG were added to the antibody–peptide reaction mixture to achieve the separation of antibody-bound tracer from free tracer. Tubers were centrifuged at 4°C and aspirated, and the radioactivity of the precipitate was determined. The percent of bound tracer, expressed as *B*/*Bo* was plotted on linear graph paper (Fig. 1); test results, in picograms per milliliter, were read directly from this calibration curve. All reagents were from Sigma Aldrich, (St. Louis, MO) unless noted.

Preparation of Radiotracer

Octreotide was iodinated by a modification of the method of Hunter and Greenwood.[14] Briefly, 3.0 μg octreotide was reacted with 2 mCi ^{125}I in the presence of chloramine T and sodium meta-bisulfite. Radio-iodinated peptide was separated from free iodine by gel filtration chromatography.

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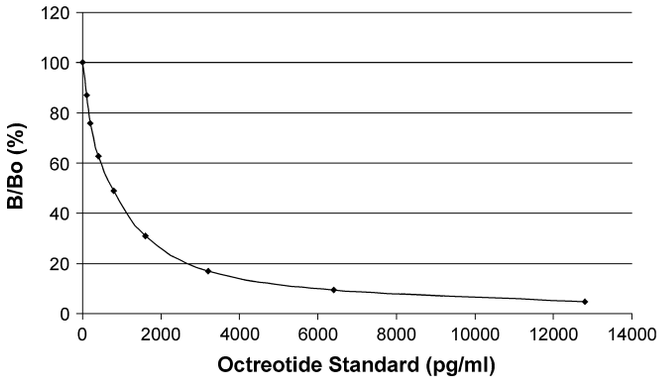


FIGURE 1. This graph depicts the plasma dose–response curve from the octreotide assay. Octreotide concentrations are in picograms per milliliter.

Sensitivity of Radioimmunoassay

The sensitivity of the current assay, at 90% *B*/*Bo*, is 100 pg/mL of sample (Fig. 1). Significantly higher sensitivity, down to 10 pg/mL or less, can be obtained through slight modification of the assay procedure.

Specificity of Radioimmunoassay

Normal serum (N = 17), normal urine (N = 10), normal saliva (N = 10), and high somatostatin-14–spiked serum, urine, and saliva specimens (up to 2560 pg of somatostatin/mL; N = 3/level; 5 levels tested) did not inhibit the binding of octreotide tracer to the antiserum. Urine (N = 10), saliva (N = 10), and serum (N = 17) samples were also devoid of any cross-reacting peptides.

Effect of Matrix on Octreotide Measurements

Octreotide standards prepared in the assay buffer, analyte free normal plasma, urine, and saliva yield almost identical dose–response curves (Fig. 2). Based on these observations, 1 calibration curve can be used for analysis of octreotide levels in plasma, serum, urine, and saliva.

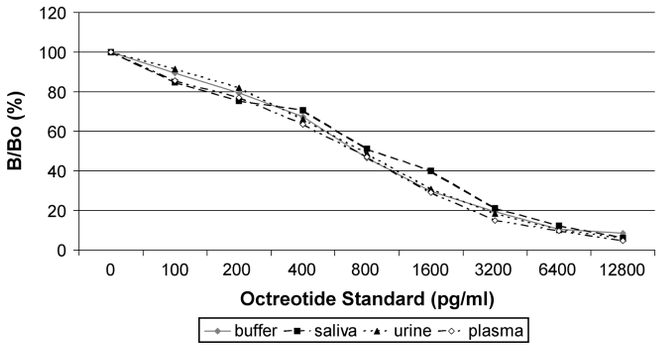


FIGURE 2. This graph depicts the dose–response curve of octreotide concentrations in urine, saliva, plasma, and assay buffer. The congruity of curves implies that this assay system is suitable for octreotide measurement in any of these matrices. The octreotide concentrations are in picograms per milliliter.

Parallelism

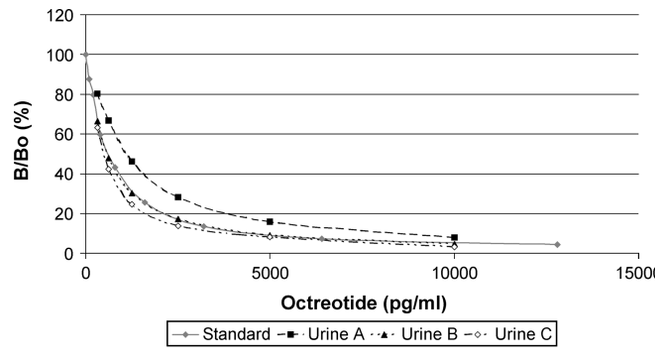
Octreotide contained in patient urine specimens yielded inhibition curves parallel to that obtained with intact, synthetic octreotide used to generate the calibration curve (Fig. 3). This suggests that there is no in vitro metabolism of octreotide and no retention of octreotide by the kidney.

Inter- and Intra-assay Variation

Interassay and intra-assay variations were tested at low (500 pg/mL), intermediate (1000 pg/mL), and high (5000 pg/mL) concentrations of octreotide. In the interassay variation assay experiment, the mean ± SD octreotide values for spiked samples were 684 ± 76.9, 1242 ± 71.2, and 5643 ± 136.9 pg/mL, respectively. The coefficients of variation for these levels were 11.2%, 5.7%, and 2.4%, respectively. Intra-assay variation was also determined at the same low, intermediate, and high concentrations of octreotide. Mean ± SD octreotide levels were 666.4 ± 110.3, 1240 ± 70.7, and 6316.5 ± 642.5 pg/mL, respectively. The coefficients of variation for these concentrations were 16.6%, 5.7% and 9.9%, respectively.

Patient Information

Patients participating in this study were part of an Internet support group for patients with neuroendocrine tumors. All patients provided information on symptoms (flushing, diarrhea, and wheezing) symptom frequency (number of episode per day and the number of days per week), and severity, height, weight, and aqueous octreotide "rescue" use patterns. Information included the number (and strength) of rescue shots used per week. This information was sent to an independent



Standards		Dilutions		Urine ID, B/Bo		
pg/ml	% B/Bo		pg/ml	A	B	C
1	100	1:10	10000	7.9	4.8	3.1
100	87.6	1:20	5000	15.8	9.2	8.1
200	79.8	1:40	2500	28.2	17.4	13.7
400	59.6	1:80	1250	46.2	30.2	24.7
800	43.3	1:160	625	66.7	47.9	42.3
1600	25.7	1:640	312	80.2	66.6	63.1
3200	13.4					
6400	7.4					
12800	4.4					

FIGURE 3. This graph shows the immunochemical identity of octreotide measured in the urine and in octreotide calibration standards used in the assay. Lines represent urinary values and multiple experiments with standards. The box (inset) shows the data used to construct these curves.

person who coded the questionnaire and individual blood, urine, and saliva specimen containers. These coded materials and a collection container were sent to the patient along with a prepaid mailer. These mailers were prepaid so that no patient identifiers were included on the box's mailing label. After the collection of the blood, urine, and saliva specimens, the mailer was expressed mailed to the laboratory at ambient temperature. All information containing patient identifiers was firewalled from the investigators and the laboratory performing the octreotide assays. Code numbers were used to identify patients and to link patient questionnaires with laboratory values. This data/specimen collection scheme was discussed with the LSUHSC's Institutional Review Board who concurred with the study design and allowed the study to be done without obtaining individual patient consents.

Patient heights and weights were recorded, and their body surface area (BSA; m^2; calculated as BSA= [height in centimeters × weight in kilograms]/3600) was determined. In addition the BMI, calculated as BMI= (weight [kg]/(height [m] × height [m]), was also calculated (Table 1).

Patient Samples

All patients fasted overnight and had their blood urine and saliva collected immediately before their next dose of LAR (trough levels). All patients stopped the use of rescue aqueous octreotide for at least 24 hours before the collection of their blood, urine, and saliva. Serum and plasma drug concentrations were expressed as octreotide levels in picograms per milliliter, picograms per milliliter per kilogram, or as octreotide (picograms per milliliter) per kilogram per milligram of LAR administered per month. LAR doses were recorded as a monthly drug dose (milligrams per month). Some patients were receiving biweekly injections of LAR, whereas others had monthly injections. Several patients (not included in this study) were receiving octreotide by continuous subcutaneous infusions, and their dose of octreotide was recorded in milligrams per day or milligrams per month. These patients are not included in the comparisons of octreotide levels in the blood urine or saliva or in the comparisons of data from this study with the data from the octreotide LAR drug registration trial.

For ease of comparison between the published results of the octreotide drug registration trial and the data collected during this study, we expressed octreotide LAR doses in milligrams of LAR administered per month. The later terminology is the conventional way that a clinician would refer to a patient's drug dose but may not be directly applicable to patients receiving aqueous octreotide by multiple daily injections or by continuous subcutaneous infusions.

Urine and nonstimulated saliva samples were also collected, and the octreotide concentration (picograms per milliliter) of these specimens was determined (Table 2). Subsequently, aliquots from each urine sample were sent to Quest Diagnostics (San Juan Capistrano, CA) for creatinine measurement, and this creatinine value was used to normalize octreotide levels for creatinine excretion (picograms of octreotide per gram creatinine excretion; Table 2).

TABLE 1. Demographic Information on Participating Patients

LAR (mg/mo)	Sex	Height (m)	Weight (kg)	BSA	BMI
20	F	1.65	73.71	1.84	27.04
20	M	1.78	108.86	2.32	34.43
20	M	1.83	86.18	2.09	25.77
20	M	1.75	88.45	2.07	28.79
20	F	1.68	48.99	1.51	17.43
20	F	1.70	79.83	1.94	27.56
20	F	1.80	83.01	2.04	25.52
20	F	1.88	113.40	2.43	32.09
30	F	1.66	142.43	2.56	51.45
30	F	1.63	68.04	1.75	25.74
30	F	1.80	95.71	2.19	29.43
30	F	1.70	122.47	2.40	42.28
30	M	1.73	79.38	1.95	26.61
30	F	1.65	61.23	1.67	22.46
30	F	1.65	72.57	1.82	26.62
30	F	1.63	58.97	1.63	22.31
30	M	1.75	65.77	1.79	21.41
30	F	1.68	86.18	2.00	30.66
30	F	1.65	48.99	1.50	17.97
30	M	1.73	95.25	2.14	31.93
30	M	1.83	77.11	1.98	23.05
30	F	1.57	71.21	1.76	28.71
30	M	1.83	86.18	2.09	25.77
30	F	1.73	74.84	1.89	25.09
30	M	1.78	79.38	1.98	25.11
30	F	1.69	90.72	2.06	31.79
30	F	1.57	52.16	1.51	21.03
40	M	1.80	99.79	2.23	30.68
40	F	1.73	70.31	1.83	23.57
53	M	1.70	62.60	1.72	21.61
60	F	1.57	47.17	1.44	19.02
60	M	1.91	77.11	2.02	21.25
60	F	1.65	55.34	1.59	20.30
60	F	1.63	72.57	1.81	27.46
60	M	1.70	72.57	1.85	25.06
60	F	1.65	58.97	1.64	21.63
60	F	1.63	57.61	1.61	21.80
60	F	1.65	133.36	2.47	48.92
60	F	1.60	48.08	1.46	18.78
60	M	1.71	62.14	1.72	21.14
67	M	1.78	106.68	2.29	33.74

Statistics

Data received from the questionnaires were inputted to an Excel spreadsheet, as were the laboratory results. The Excel spreadsheet was imported into SPSS for Windows (version 11; SPSS) for statistical analyses. Summary statistics were calculated and correlation, and regressions analyses were performed.

RESULTS

Fifty-two patients who were on stable doses of LAR for 3 or more months were recruited from the www.yahoo.com carcinoid group. These patients sent in clinical information or

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TABLE 2. Octreotide Level in Plasma, Saliva, and Urine Sorted by Daily Dose of Octreotide in ISI Trial

LAR: (mg/mo)	Plasma (pg/mL)	Saliva (pg/mL)	Urine (pg/mL)	Urine (μg/mL/g creatinine)
20	3,946	41	93,760	142
20	2,108	48	37,491	36
20	1,691	69	34,267	ND
20	4,235	ND	67,549	ND
20	2,628	ND	94,833	ND
20	2,049	ND	13,185	53
20	1,655	53	156,751	ND
20	1,831	11	77,500	ND
30	8,288	42	270,851	ND
30	3,512	285	124,250	259
30	3,087	155	70,100	260
30	5,685	76	245,394	ND
30	5,347	280	206,500	217
30	7,153	369	61,251	170
30	4,626	73	125,283	ND
30	1,746	ND	ND	ND
30	8,418	89	99,176	134
30	3,339	296	101,737	127
30	8,142	451	129,993	351
30	5,318	134	193,490	ND
30	3,782	145	72,550	119
30	2,593	73	316,800	117
30	3,339	77	139,635	116
30	13,937	ND	213,582	ND
30	2,288	28	43,013	53
30	2,390	33	93,460	123
30	6,581	76	226,904	ND
40	2,820	50	150,696	ND
40	4,177	193	321,843	156
53	9,027	147	72,425	ND
60	8,003	321	204,960	285
60	8,554	64	676,618	274
60	14,435	1,013	334,500	683
60	9,509	187	843,544	315
60	11,158	164	136,150	164
60	23,541	92	777,377	ND
60	4,052	ND	203,568	ND
60	11,544	119	79,972	250
60	11,938	216	540,780	ND
60	6526	ND	433,666	ND
67	3,433	91	336,166	187

ND, not done.

TABLE 3. Correlation Between Plasma and Serum Octreotide as Function of LAR Dose

Sample	Dose (mg/mo)	Mean Octreotide (pg/mL)	SD	Correlation Factor (*R*)
Plasma	20	2,518	1020	0.91
	30	5,241	3005	0.98
	60	10,926	5330	0.96
Serum	20	2,466	906	
	30	5,452	3339	
	60	10,248	4810	

serum, plasma, urine, and saliva to Inter Science Institute for octreotide analysis (Tables 1–3). Plasma octreotide levels and corresponding patient weights (kilograms) from patients receiving 10, 20, or 30 mg LAR/mo on the octreotide drug registration trial were kindly supplied by Novartis Pharmaceutical. Table 1 shows the demographic information on this group of patients. Height and weight are measured values. Drug dose was tabulated as the number of milligrams of octreotide used per day. This data was expressed in this fashion to account for variations in drug administration schedules. BMI and surface area (meters squared) are calculated values. Data are also shown for the number of milligrams of LAR used per month for ease of comparison between the Novartis octreotide registration trial and the ISI trial. Table 1b shows the octreotide levels (picograms per milliliter) in plasma, saliva, and urine sorted by the daily dose of octreotide used in the ISI trial. Urinary octreotide levels were subsequently corrected for the amount of creatinine in the urine (grams per milliliter) and expressed as picograms of octreotide per gram of urinary creatinine. Table 3 shows the correlation between plasma and serum octreotide levels as a function of LAR dose in milligrams per month.

ISI Data

The ISI octreotide assay used in this study seems to be highly sensitive, highly specific, and can provide accurate information on octreotide concentrations in plasma, serum, saliva, and urine (Figs. 1–3). Fifty-two patients sent in information or serum, plasma, and urine specimens. Forty-one patients received LAR therapy at a stable dose for 3 or more months, sent in complete clinical information, and had plasma and serum specimens available for analysis. Only patients receiving LAR are reported in this study. Patients receiving octreotide by continuous subcutaneous infusion were not included in this report. Patients were sorted by their monthly dose of LAR. Eight received 20 mg of LAR per month, 19 received 30 mg/mo of LAR, and 10 received LAR at doses of 60 mg/mo. Four other patients reported receiving other doses per month (2 at 40 mg/mo, 1 at 53 mg/mo, 1 at 67 mg/mo). Data from these 41 patients were used in correlation and regression analyses.

Plasma and serum octreotide levels were essentially identical in this prospective trial (Table 3). The mean ± SD plasma octreotide levels for patients receiving 20, 30, or 60 mg/mo LAR doses were 2518 ± 1020, 5241 ± 3004, and 10,925 ± 5330 pg/mL, respectively (Table 4). Patients receiving LAR at doses of 60 mg/mo reported no significant side effects from their high-dose LAR therapy.

There was a dose-dependent ($r = 0.60$, $P < 0.01$) increase in plasma octreotide levels when the patient's octreotide dose (20, 30, or 60 mg/mo; ISI data) was plotted against the mean plasma octreotide level (Table 4).

The patient weights were relatively consistent among the LAR dose groups in the ISI trial and between the Novartis and ISI trials (Table 5). We also expressed octreotide levels as picograms per milliliter per kilogram patient weight and picograms per milliliter per kilogram per milligram of LAR

TABLE 4. Effect of Increasing Monthly Octreotide LAR Dose on Octreotide Level in Novartis and ISI Studies

	Novartis Study			ISI Study		
Dose	Mean OA (pg/mL)	SD	N	Mean OA (pg/mL)	SD	N
10	1154	748	21	ND	ND	ND
20	1914	972	20	2,518	1020	8
30	4247	2733	24	5,241	3004	19
60	ND	ND	ND	10,925	5330	10

OA, octreotide acetate; ND, not done.

TABLE 5. Mean Body Weight in Novartis and ISI Studies

	Novartis Study			ISI Study		
Dose	Mean Weight (kg)	SD	N	Mean Weight (kg)	SD	N
10	77.1	15.9	21	ND	ND	ND
20	75.1	17.1	20	85.3	20.2	8
30	78.0	11.8	24	80.5	24	19
60	ND	ND	ND	68.6	24	10

ND, not done.

administered per month (Table 6). These data also follow a dose-dependent trend.

Urine and nonstimulated saliva samples were collected, and the octreotide concentrations were expressed as micrograms per milliliter of octreotide in saliva and urine. Subsequently, urinary creatinine was also measured in these specimens, and the urinary octreotide concentration was expressed as picograms of octreotide per gram of urinary creatinine (Table 2). There was a positive direct correlation between urinary octreotide levels (expressed as micrograms per gram creatinine) and saliva levels with plasma octreotide levels ($P = 0.01$). Pearson correlation coefficients were calculated for the relationships between LAR dose and octreotide levels in the urine, plasma, serum, and saliva (Table 7). Correlation coefficients between urine plasma serum and saliva octreotide levels were also calculated and showed that there was a positive correlation between plasma octreotide levels and the octreotide levels in urine, serum ($P < 0.01$, respectively), and saliva ($P < 0.05$).

Combined Novartis and ISI data

Data on patient octreotide levels and corresponding patient weight, obtained during the drug registration trial, was kindly provided by Novartis Pharmaceuticals. We compared the Novartis data to the data from this prospective ISI Internet-based trial to determine if both sets of data were consistent. Octreotide levels in patients receiving 20- or 30-mg doses of LAR in both trials were statistically the same (ANOVA, $P =$ not significant, respectively; Table 4).

In the combined series, 21 patients received octreotide at doses of 10 mg/mo (Novartis data). In the combined Novartis/ISI data sets, 28 patients received LAR at doses of 20 mg/mo and 43 patients received LAR at a dose of 30 mg/mo. In the ISI study, 10 patients received octreotide at doses of 60 mg/mo of LAR (Table 4).

The mean weights in all of these groups were comparable. The mean weight was 77.1 ± 15.9 (SD) kg in the LAR 10 mg/mo Novartis drug registration trial. In patients receiving LAR at doses of 20 or 30 mg/mo (combined data), their mean weights were 78.0 ± 18.3 and 79.1 ± 17.4 kg for LAR 20- and 30-mg monthly doses, respectively. The mean weight of the group of patients receiving LAR at a dose of 60 mg/mo (ISI data) was 74. 7± 28.7 kg (Table 5).

The mean plasma levels of octreotide in patients receiving LAR at a dose of 10 mg/mo in the Novartis registration trial was 1153 ± 748 pg/mL. No ISI data are available for patients on 10 mg/mo of LAR (Table 4). In the combined study using the Novartis and ISI data sets, the mean plasma levels of octreotide for patients receiving 20 and 30 mg/mo, respectively, were 2087 ± 1005 and 4686 ± 2865 pg/mL (Table 4). In the ISI series, patients also had plasma octreotide levels measured while on octreotide LAR at doses of 60 mg/mo (n = 10). The mean plasma octreotide levels in patients receiving LAR at 60 mg/mo were 10,925 ± 5330 pg/mL (Table 4).

We also tabulated the combined data from our trial and the registration trial and examined the effect of patient weight (kilograms) on plasma octreotide levels in patients receiving octreotide therapy at various doses (milligrams per month administered; Table 6). These data imply that, as the monthly dose of octreotide increases, blood levels of the drug increase, even when corrected for body weight and the number of milligrams of LAR administered per month. This increase follows a relatively linear trend (Table 6).

Figure 4 further explores the combined data. The range of plasma octreotide levels is plotted versus the octreotide LAR doses. The dark line on each box plot represents the median, whereas the box shows the range of the 25th to 75th

TABLE 6. Effect of Body Weight and Monthly Dose on Circulating Plasma Octreotide Level

LAR Dose (mg/mo) Administered (combined data)	N (combined data)	Weight (kg ± SD)	Plasma Octreotide (pg/mL ± SD)	Octreotide (pg/mL/kg)	Octreotide (pg/mL/kg/mg of LAR administered per month)
10	21	77.1 ± 15.9	1,153 ± 748	16.2	1.6
20	28	78.0 ± 18.3	2,087 ± 1,006	28.7	1.4
30	43	79.1 ± 17.4	4,686 ± 2,865	64.7	2.1
60	10	74.7 ± 28.7	10,926 ± 5,330	173.6	2.9

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TABLE 7. Pearson Correlation Coefficients for Relationship Between LAR Dose and Octreotide Levels in Urine, Plasma, Serum, and Saliva

	LAR (mg/mo)	Plasma (pg/mL)	Serum (pg/mL)	Urine (μg/mL/g creatinine)	Saliva (pg/mL)
LAR (mg/mo)	1	0.575†	0.595†	0.550†	0.302
Plasma (pg/mL)	0.575†	1	0.974†	0.740†	0.365*
Serum (pg/mL)	0.595†	0.974†	1	0.665†	0.320
Urine (μg/mL/g creatinine)	0.550*	0.740†	0.665†	1	0.854†
Saliva (pg/mL)	0.302	0.365*	0.320	0.854†	1

*Significant at the 0.05 level (2-tailed).
†Significant at the 0.01 level (2-tailed).

percentiles. The two reference lines are drawn at the K_d and saturation levels for the sst2 receptor.

To further explore the relationship between study variables, a multiple linear regression analysis was performed. Because the Novartis study only reports patient weight, we included the independent variables of LAR dosage per month and patient weight to predict the plasma octreotide level. The results of analysis showed that both LAR dosage contributed positively and weight contributed negatively to the regression equation ($R^2 = 0.5$, $P < 0.05$), with LAR dosage being 5 times more sensitive than weight (standardized coefficients, $\beta = 0.69$ for LAR and -0.15 for weight).

We correlated the plasma octreotide levels of patients receiving 20, 30, or 60 mg/mo of LAR with the symptom severity scores for flushing and diarrhea (episodes per day × the number of days per week). There was no statistically significant correlation between plasma octreotide level and the number of stools or flushing episodes per week. Many of these patients had been previously treated with lower doses of LAR. Theoretically this lack of correlation might be caused by the use of higher doses of LAR in those patients with the highest symptom severity or frequency.

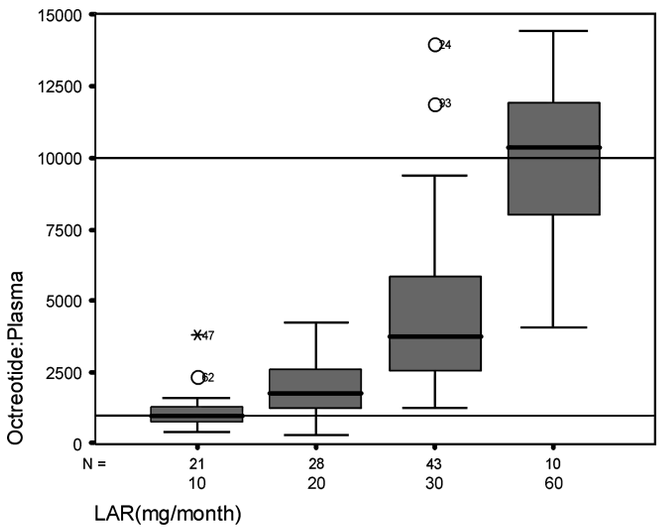

FIGURE 4. This figure represents the range of plasma octreotide levels versus the monthly dosage of LAR for the combined Novartis and ISI data. The dark line on each box-plot represents the median of the range, whereas the bottom and top of the solid box represents the 25th and 75th percentile of the plasma levels. The 2 reference lines are drawn at the K_d and saturation levels of the sst2 receptor. Only the 60 mg/mo LAR dose covers the concentrations that would saturate sst2. Circles and asterisks with values represent data that is out of range.

Two patients started their initial LAR therapy at a dose of 10 mg/mo, whereas 31 patients started LAR therapy at 20 mg/mo. Eight patients started LAR therapy at 30 mg/mo. The mean length of time on LAR therapy was 30 ± 17 months, and the average number of months on the current dose was 17 ± 14 months. This implies that most of patients started on LAR will have to have their dose adjusted upward over time. In this study, a total of 71% of patients had an upward dose adjustment over time. This need for progressively higher doses of LAR may reflect the development of tachyphylaxis, down-regulation of receptor number, tumor growth, or increased amine production from the tumor.

At the time of this survey, 38% of patients felt their symptoms were well controlled, and 47% felt their symptoms were fairly well controlled. Twelve percent felt their symptoms were not well controlled, and 3% of respondents did not respond to these symptom severity and frequency questions.

Subcutaneous aqueous octreotide was used as rescue medication by patients in all LAR dose groups. The number of rescue injections used per symptomatic episode was equally divided between 1, 2, and 3 injections per episode. There was no correlation between the use of rescue medication and the plasma octreotide levels.

DISCUSSION

The K_d of octreotide for sst2 is approximately 1 nmol/L (1000 pg/mL).[2,3] These levels are achieved in patients receiving LAR doses of 20 mg/mo. Patients receiving LAR at doses of 30 mg/mo have mean plasma octreotide levels (≅5000 pg/mL, 5 nmol/L) that exceed the K_d of octreotide for sst2 by 5-fold but do not reach levels that completely saturate this receptor. In contrast, patients who are given LAR at doses of 60 mg/mo achieve mean plasma levels (≅11,000 pg/mL) that should completely saturate the sst2 receptor. At least theoretically, those patients with sst2-saturating levels of octreotide in their plasma should have achieved maximal clinical benefit from the use of octreotide acetate. This is consistent with the work of Anthony et al,[13] who showed an excellent safety profile and limited toxicity of high-dose octreotide therapy. In

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the study of Anthony et al, patients derived little additional antitumor or symptom control benefit from extremely high doses of octreotide. Whereas the plasma octreotide levels achieved in patients receiving LAR at doses of 60 mg/mo are adequate to saturate sst2, these levels may not saturate other sst's (with lower affinity for octreotide) that may contribute to the clinical response. Saturation of these receptors will require higher levels of octreotide. The role of these other sst's in the overall clinical response may be better defined by studies of SOM-230, a somatostatin analog that avidly binds to sst1, 2, 3, and 5.[15] Preliminary results of SOM-230 used in patients with carcinoid syndrome who failed octreotide therapy are encouraging (L. Anthony, personal communication).

The results of our analysis showed that LAR dosage contributed positively and weight contributed negatively to the regression equation ($R^2 = 0.5$, $P < 0.05$), with LAR dosage being 5 times more sensitive than weight (standardized coefficients $\beta = 0.69$ for LAR and -0.15 for weight). These observations imply that, at a given LAR dose, a patient who is heavier will have relatively lower plasma octreotide levels than a significantly lighter patient. This has led some investigators to use starting LAR doses of 20 mg/mo in patients less than 70 kg and a starting dose of 30 mg/mo in patients greater than 70 kg. Whereas weight plays a significant role in the plasma concentrations of LAR, individual variations in plasma octreotide levels (at a given LAR dose) are great enough that measurement of plasma octreotide levels in patients with suboptimal symptom control is warranted after achievement of steady-state levels of this drug (usually 3 months on the same dose of LAR).[5]

The octreotide assay developed for this study seems to be highly sensitive and yields octreotide levels consistent with those seen in the LAR drug registration trial.[5] In our study, we extended the dose range of LAR to include patients dosed at 60 mg/mo. At these doses, we showed that plasma or serum concentrations were achieved that, in vitro, would completely saturate the sst2 receptor. Plasma and serum octreotide levels from the same patient appear to be nearly identical, implying that this drug is highly stable and resistant to degradation in these matrices. In addition, urine and salivary octreotide levels are measurable, although their values are higher (urine) or lower (saliva) than the respective patient-matched plasma or serum values. Urine, saliva, plasma, and serum specimens, spiked with known quantities of octreotide, yielded identical binding curves. This implies that all 4 of these matrices are valid for the measurement of octreotide levels. The use of urinary or salivary octreotide measurements remains unknown; however, the positive correlation between plasma octreotide levels and octreotide levels in the urine and saliva might allow one to speculate that measurements of these levels might be useful for self-monitoring of octreotide drug levels on a more frequent basis.

Measurement of plasma octreotide acetate levels may be helpful in patients receiving octreotide acetate therapy, especially when the clinician is faced with a patient with increasing symptoms not adequately controlled by frequent administration of rescue medication or a patient with progressive tumor growth. In these cases, increasing a patient's drug dose may be indicated. Approximately 70% of patients in this study had previously received LAR at lower doses. The reason that an individual patient received a specific dose of LAR was not discernible in this study, but one can assume that at least some of these patients were given a higher LAR dose because of a lack of adequate symptomatic control at the lower LAR dose. The potential causes for the progressive loss of symptom control are highly controversial but include the development of tachyphylaxis, an increased volume of tumor, an increase in the production of amines per a given volume of tumor, or the down-regulation of sst2 receptor number.

Interval measurement of octreotide levels may help determine if a patient has had a sporadic "bad injection" (often caused by clogging of the administration needle or too superficial injection of the medication) or needs more medication on a chronic basis. Commonly, patients receiving 10, 20, or 30 mg of Sandostatin® per month may experience symptoms that are not adequately controlled by their monthly injections. In these cases, use of rescue medication (defined as 3 times a day aqueous octreotide use [100–500 μg/dose] for 1 day) is indicated. In the drug registration trial, regardless of the dose of octreotide administered (10, 20, or 30 mg LAR/mo), approximately 40% of patients used rescue medication several times a week. Currently, the FDA has approved this medication at maximum monthly doses of 30 mg. However, in a recent study by Anthony et al,[6] 20% to 40% of patients on chronic LAR therapy required monthly LAR doses higher than the FDA-approved doses to control symptoms. In our study, patients receiving LAR at doses of 60 mg/mo tolerated these higher drug doses without any unusual side effects. Clearly, the ability to obtain plasma octreotide levels will help guide clinical decision-making in patients with symptoms that are difficult to control.

Alternatively, in patients experiencing tumor growth, independent of their symptom control, the clinician may choose to increase octreotide doses in the hope that this will further suppress tumor progression. Whereas the antitumor effect of octreotide remains controversial and may be dose-dependent, the safety profile of octreotide is well established and is not dose-dependent. This safety profile allows the clinician a great deal of latitude in choosing an appropriate octreotide dose.

Octreotide acetate binds to the sst2 receptor with an affinity (K_d) of approximately 1×10^{-9} mol/L (≅1000 pg/mL), but higher (≅10,000 pg/mL or 1×10^{-8} mol/L) concentrations of octreotide are required to completely saturate this receptor. Doses of LAR of 10 or 20 mg/mo achieve plasma levels that approximate the K_d for the sst2 receptor. LAR, when given at a dose of 30 mg/mo, results in plasma levels that exceed the K_d for the sst2 receptor by 5-fold but does not achieve the plasma concentrations that will completely saturate the sst2 receptor. This may explain the need for frequent administration of rescue medication or the frequency of use of doses of LAR that exceed the 30 mg/mo FDA guidelines. Administration of LAR, at doses of 60 mg/mo, appears to be safe and achieves plasma levels that should completely saturate sst2. The starting dose of LAR needs to be chosen based on the patients' weight to achieve optimum octreotide levels. Frequent measurement of octreotide levels may be useful to guide octreotide therapy in patients with poorly controlled symptoms or those experiencing tumor growth during LAR therapy.

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